Società Chimica Italiana

Programma del
XXVI Congresso Nazionale
della Società Chimica Italiana

Centro Congressi Hotel Ariston
Paestum (SA), 10-14 settembre 2017

➢Divisione di Chimica Analitica

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DIVISIONE DI CHIMICA ANALITICA
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Programma Scientifico

Divisione di Chimica Analitica

Lunedi' 11 Settembre 2017

	Sala Paestum A
	Conferenza Plenaria: Chimica Analitica
	Chair: Aldo Roda
9.00 – 9.15	*Apertura Congresso della Divisione Chimica Analitica*
9.15 – 10.00	ANA-PL01: Luisa Torsi *Printable organic bioelectronics for bioanalytical detections at the physical limit: is this feasible?*
10.00 – 10.30	*Conferenza Vincitore del Premio Giovane Ricercatore* ANA-IN01: Susy Piovesana *Cutting-edge developments in shotgun proteomics, peptidomics and shotgun phosphoproteomics in real matrices*
10.30 – 11.00	Coffee Break
	Sessione Parallela: Scienza delle Separazioni
	Chair: Maria Careri
11.00 – 11.20	*Finalista premio Levi* ANA-IN02: Anna Laura Capriotti *A new magnetic graphitized carbon black TiO$_2$ composite for phosphopeptide selective enrichment in shotgun phosphoproteomics*
11.20 – 11.50	ANA-KN01: Sara Bogialli, Paolo Pastore, Luca Lucentini *High Resolution mass spectrometry: the re-evolution of the risk assessment and management*
11.50 – 12.10	ANA-OR01: Tonino Caruso *A validated SIM GC/MS method for the simultaneous determination of linear polyalkylsiloxanes OH-terminated in ctamethylcyclotetrasiloxane*
12.10 – 12.30	ANA-OR02: Giorgia La Barbera, Giulia Praticò, Göezde Gurdeniz, Maj Britt Schmidt Andersen, Lars Ove Dragsted *Identification of urinary biomarkers of meat and dairy products intake by means of UHPLC-QTOF/MS untargeted metabolic profiling*
12.30 – 12.50	ANA-OR03: Massimo Del Bubba, Lapo Renai, Claudia Ancillotti, Lorenzo Ciofi, Daniele Rossini, Leonardo Checchini, Maria Concetta Bruzzoniti, Serena Orlandini, Donatella Fibbi, Sandra Furlanetto *On-line SPE-LC-MS/MS analysis of wide-range polarity pharmaceutical compounds in aqueous matrices: problems and solutions*

	Sala Olimpia
	Sessione Parallela **Bioanalitica**
	Chair: **Mara Mirasoli**
11.00 – 11.30	ANA-KN02: Zangheri Martina*,* Mara Mirasoli, Laura Anfossi, Fabio Di Nardo, Cristina Giovannoli, Massimo Guardigli, Claudio Baggiani, Aldo Roda *Biosensors based on chemiluminescent-Lateral Flow Immunoassay: a powerful approach for integrated analytical platforms*
11.30 – 11.50	ANA-OR04: Nicola Marchetti, Caterina Bergantin, Annalisa Maietti, Luisa Pasti, Alberto Cavazzini *Insights into structure-bioaccessibility relationships of polyphenols in red chicory by HPLC-MS/MS*
11.50 – 12.10	ANA-OR05: Marcello Manfredi, Chiara Guglielmetti, Maria Mazza, Sonia Brusadore, Paolo Giuseppe Ubaldi, Luca Magnani, Stefano Gili, Emilio Marengo, Pier Luigi Acutis *Shotgun Proteomics for Detecting Seafood Fraud*
12.10 – 12.30	ANA-OR06: Matteo Crosera, Marcella Mauro, Elena Baracchini, Massimo Bovenzi, Francesca Larese Filon, Gianpiero Adami *In vitro meningeal permeation of $MnFe_2O_4$ Nanoparticles*
12.30 – 12.50	ANA-OR07: Camilla Montesano, Gabriele Vannutelli, Federico Fanti, Flaminia Vincenti, Roberta Curini, Adolfo Gregori, Anna Rita Togna, Matteo Marti, Isabella Canazza, Manuel Sergi *Identification of MT-45 metabolites: in silico prediction, in vitro incubation with rat hepatocytes and in vivo confirmation*

	Sala Penelope
	Sessione Congiunta: **Chimica Analitica - Elettrochimica - Gruppo Sensori**
	Chair: **Aldo Roda**
11.00 – 11.30	ANA/ELE/GS-KN01: Fabiana Arduini, Stefano Cinti, Giorgio Scordo, Renato Massoud, Danila Moscone, Giuseppe Palleschi *New Sustainable and cost-effective paper-based electrochemical (bio)sensors*
11.30 – 11.50	ANA/ELE/GS-OR01: Erika Scavetta, Federica Mariani, Isacco Gualandi, Domenica Tonelli, Marta Tessarolo, Beatrice Fraboni *All PEDOT:PSS electrochemical transistors as a platform for sensing*
11.50 – 12.10	ANA/ELE/GS-OR02: Alice Soldà, Marco Giorgio, Pier Giuseppe Pelicci, Nicholas Dale, Francesco Paolucci, Stefania Rapino *Development of enzyme-based microsensors for ex vivo analyses*
12.10 – 12.30	ANA/ELE/GS-OR03: Ornella Abollino, Andrea Ruo Redda, Francesca Durbiano, Eleonora Conca, Mery Malandrino, Agnese Giacomino *Potential and limitations of voltammetric measurements for the characterization of electrode surface*
12.30 – 12.50	ANA/ELE/GS-OR04: Laura Fabiani, Giulia Volpe, Elisabetta Delibato, Eleonora Pucci, Silvia Piermarini, Giuseppe Palleschi *Development of an electrochemical magneto-immunosensor for the detection of Campylobacter: a preliminary study*

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	Sala Athena
**"La Trasversalità della Chimica nei Beni Culturali"  ** *Sessione Congiunta: Chimica Analitica -Fisica - Chimica dell'Ambiente e dei Beni Culturali*

	"La Trasversalità della Chimica nei Beni Culturali" *Sessione Congiunta: Chimica Analitica -Fisica - Chimica dell'Ambiente e dei Beni Culturali*
*Chair: Lucia Toniolo*
9.00 – 9.25	ABC/ANA/FIS-KN01: Rocco Mazzeo, Stjin Legrand, Giorgia Sciutto, Silvia Prati, Emilio Catelli, Paolo Oliveri, Koen Janssens *Macro Mid-Infrared total reflection (MA-FTIR) mapping for the characterization of outdoor bronze surfaces*
9.25 – 9.40	ABC/ANA/FIS-OR01: Rachele Elisabetta Camerini *Silica-based composites for the consolidation of earthen materials*
9.40 – 9.55	ABC/ANA/FIS-OR02: Pamela Ferrari *Chemical poly ethyl methacrylate (PEMA) organogels for the removal of pressure sensitive tapes (PSTs) from paper artworks*
9.55 – 10.10	ABC/ANA/FIS-OR03: Nicole Bonelli *Surface cleaning of artworks: structure and dynamics of nanostructured fluids confined in a polymeric network*
10.10 – 10.25	ABC/ANA/FIS-OR04: Giovanna Poggi *Nanocomposites for the consolidation and deacidification of cellulose-based artifacts*
10.30 – 11.00	Coffee Break

	Sessione Congiunta: Chimica Analitica -Fisica - Chimica dell'Ambiente e dei Beni Culturali
*Chair: Luigia Sabbatini*
11.00 – 11.25	ABC/ANA/FIS-KN02: Erika Ribechini, Laura Blanco-Zubiaguirre, Ilaria Degano, Jacopo La Nasa, Javier García-Iñañez, Maitane Olivares, Kepa Castro *GC/MS and HPLC/MS characterization of organic residues from ceramic vessels of Basque whalers of the 16th to 17th century*
11.25 – 11.40	ABC/ANA/FIS-OR05: Francesca Di Turo, Maria Teresa Doménech-Carbò, Noemì Montoya, Caterina De Vito, Fiorenzo Catalli, Gabriele Favero, Antonio Doménech-Carbò *FIB-FESEM-EDX study of silver Roman coins: characterization of the core microstructure and corrosion products with a multi-analytical approach*
11.40 – 11.55	ABC/ANA/FIS-OR06: Antonella Casoli, Clelia Isca, Stefano Volpin *I materiali e la tecnica pittorica di Konrad Witz nel suo capolavoro di Ginevra*
11.55 – 12.10	ABC/ANA/FIS-OR07: Elettra Barberis, Marcello Manfredi, Simone Baiocco, Eleonora Conte, Fabio Gosetti, Elisa Robotti, Pier Giorgio Righetti, Emilio Marengo *Towards the Non-invasive Protemic Analysis of Cultural Heritage and Archaeological Objects*
12.10 – 12.25	ABC/ANA/FIS-OR08: Giuseppe Egidio De Benedetto, Antonio Pennetta, Daniela Fico *Provenance of Bitumen from different Apulian Bronze Age settlements through a biomarker based approach*
12.25 – 12.40	ABC/ANA/FIS-OR09: Marzia Fantauzzi, Federica Cocco, Bernhard Elsener, Gabriele Navarra, Antonella Rossi *From XAES signals to depth-profile reconstruction: the case of copper and copper alloys*
12.40 – 13.00	ABC/ANA/FIS-OR10: Marco Orlandi, Luca Zoia, Anika Salanti, Diego Tamburini, Jeannette Jacqueline Łucejko, Francesca Modugno, Maria Perla Colombini *Integrated approach for the chemical characterization of archaeological woods*

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13.00 – 14.00	Intervallo Pranzo

	Sala Paestum B
14.00 – 15.00	*Sessione Poster 1: Beni culturali, Ambiente, Equilibri (ANA PO01-PO40)*
	Sala Paestum A

	Sessione Congiunta: ***Chimica Analitica - Elettrochimica - Gruppo Sensori***
	Chair: ***Salvatore Daniele***
15.00 – 15.30	ANA/ELE/GS-KN02: Andreas Lesch, Milica Jovic, Victor Costa Bassetto, Hubert H. Girault *Preparing Nanoparticles on Large Electrodes Using Inkjet Printing and Flash Light Irradiation*
15.30 – 15.50	ANA/ELE/GS-OR05: Stefania Rapino, Mirella Trinei, Alice Soldà, Marco Giorgio, Luca Bartolini, Matteo Iurlo, Massimo Marcaccio, Francesco Zerbetto, Pier Giuseppe Pelicci, Francesco Paolucci *Rapid, Low-cost and Portable Electrochemical Assay for Heart Injury Diagnosis*
15.50 – 16.10	ANA/ELE/GS-OR06: Maria Rita Tomei, Daniela Neagu, Fabiana Arduini, Danila Moscone *Carbon black modified screen-printed electrodes to detect chlorine dioxide.*
16.10 – 16.30	ANA/ELE/GS-OR07: Vincenzo Mazzaracchio, Daniela Neagu, Alessandro Porchetta, Giuseppe Palleschi, Daniela Moscone, Alice Pomponi, Giovanni Faggioni, Florigio Lista, Fabiana Arduini *Bacillus anthracis spore detection by using a label free impedimetric aptasensor*
16.30 – 17.00	Coffee Break
	Sessione Parallela: ***Ambiente***
	Chair: ***Claudio Minero***
17.00 – 17.30	ANA-KN03: Silvia Becagli, Fabrizio Anello, Carlo Bommarito, Federico Cassola, Giulia Calzolai, Tatiana Di Iorio, Alcide di Sarra, Jose-Luis Gómez-Amo, Franco Lucarelli, Daniela Meloni, Silvia Nava, Giandomenico Pace, Mirko Severi, Damiano Massimiliano Sferlazzo, Rita Traversi, Roberto Udisti *Multidisciplinary approach to constrain the ship contribution to the aerosol of the Central Mediterranean Sea*
17.30 – 17.50	ANA-OR08: Noemi Colozza, Fabiana Arduini, Danila Moscone, Giuseppe Palleschi *A reagent-free paper-based biosensor for the detection of mustard agents*
17.50 – 18.10	ANA-OR09: Eleonora Conca, Mery Malandrino, Agnese Giacomino, Andrea Ruo Redda, Sandro Buoso, Marco Grotti, Ornella Abollino, Roberto Udisti *Development of a suitable sequential extraction procedure for the assessment of the availability and reactivity of metal species in Arctic PM10 samples*
18.10 – 18.30	ANA-OR10: Marianna Rossetti, Alessandro Porchetta, Francesco Ricci, Giuseppe Palleschi *DNA-based single step fluorescence detection of Domoic Acid in marine water*
18.30 – 20.00	***Assemblea della Divisione di Chimica Analitica***

	Sala Olimpia
	*Sessione Parallela: **Scienza delle separazioni***
	*Chair: **Alberto Cavazzini***
15.00 – 15.30	ANA-KN04: Paola Donato, Veronica Inferrera, Daniele Giuffrida, Francesco Cacciola, Paola Dugo, Luigi Mondello *Supercritical fluid chromatography × reversed-phase liquid chromatography with quadrupole time-of-flight and ion mobility mass spectrometry detection: a proof of concept*
15.30 – 15.50	ANA-OR11: Carmela Maria Montone, Anna Laura Capriotti, Chiara Cavaliere, Riccardo Zenezini Chiozzi, Aldo Laganà *Development of an analytical strategy for the purification and identification of bioactive peptides from microalgae*
15.50 – 16.10	ANA-OR12: Martina Catani, Omar H. Ismail, Simona Felletti, Francesco Gasparrini, Alberto Cavazzini *Investigation of mass transfer phenomena in new core-shell and sub-2μm fully porous chiral stationary phases for ultrafast high-performance enantioseparations*
16.10 – 16.30	ANA-OR13: Valentina Marassi, Barbara Roda, Andrea Zattoni, Sonia Casolari, Pierluigi Reschiglian *Integration of separation-based analytical platforms in the development of nanomaterials as bioactive products*
16.30 – 17.00	Coffee Break
	*Sessione Parallela: **Equilibri in soluzione e speciazione***
	*Chair: **Concetta De Stefano***
17.00 – 17.20	ANA-OR14: Emilia Furia, Rosangela Elliani, Luana Malacaria, Antonio Tagarelli *Coumarin-3-carboxylic acid as ligand: acid-base properties, interactions with Fe3+and characterization of complexes in aqueous solution*
17.20 – 17.40	ANA-OR15: Denis Badocco, Valerio Di Marco, Alfonso Venzo, Marco Frasconi, Paolo Pastore *Ion pair formation between tertiary aliphatic amines and perchlorate in the biphasic H_2O / CH_2Cl_2 system*
17.40 – 18.00	ANA-OR16: Clemente Bretti, Rosalia Maria Cigala, Francesco Crea, Concetta De Stefano, Giuseppe Gattuso, Anna Irto, Gabriele Lando, Demetrio Milea, Silvio Sammartano *Acid-base and chelating properties of Gantrez copolymers*
18.00 – 18.20	ANA-OR17: Gaetano De Tommaso, Mauro Iuliano, Lucia De Rosa, Luca Domenico D'Andrea, Alessandra Romanelli, Gaetano Malgieri, Carla Isernia *Coordination of a bis-histidine oligopeptide with Ga^{3+} ion in aqueous solution*

	Sala Cassandra
	*Sessione Congiunta: **Chimica analitica - Spettrometria di massa***
	*Chair: **Tommaso Cataldi***
15.00 – 15.30	ANA/MAS-KN01: Cosima Damiana Calvano, Marco Glaciale, Sara Granafei, Anna Maria Sardanelli, Luana Bellanova, Antonella Mastrorocco, Francesco Palmisano, Tommaso Cataldi *Advanced mass spectrometric techniques for the untargeted lipidome characterization of fibroblasts in early on-set Parkinson's disease patients*
15.30 – 15.50	ANA/MAS-OR01: Simone Nicolardi, Yuri E.M. van der Burgt, Jeroen D.C. Codée, Manfred Wuhrer, Cornelis, H. Hokke, Fabrizio Chiodo

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	Structural characterization of bio-functionalized gold nanoparticles by ultrahigh resolution mass spectrometry
15.50 – 16.10	ANA/MAS-OR02: Lucia Cenci, Graziano Guella, Alessandra Maria Bossi *Molecularly imprinted materials coupled to MALDI-TOF Mass Spectrometry for the targeted analysis of peptides and proteins*
16.10 – 16.30	ANA/MAS-OR03: Rossana Scarpone, Roberta Rosato, Federico Bacá, Manuel Sergi, Dario Compagnone *Unknown and non-target analysis to determine pesticides in fruit and vegetables by means of UHPLC-HRMS (Orbitrap)*
16.30 – 17.00	Coffee Break
*Sessione Congiunta: **Chimica analitica – Spettrometria di Massa***	
*Chair: **Paola Montoro***	
17.00 – 17.30	ANA/MAS-KN02: Danilo Sciarrone, Antonino Schepis, Luigi Mondello *Advanced analytical capabilities exploiting isotope ratio mass spectrometry and quadrupole mass spectrometry coupled to multidimensional gas chromatography*
17.30 – 17.50	ANA/MAS-OR04: Andreina Ricci, Paola Cimino, Anna Troiani, Federico Pepi, Stefania Garzoli, Chiara Salvitti and Vincenzo Barone *From ascorbic acid to furan molecules: a theoretical and experimental study on the gas phase acid catalyzed degradation of vitamin C*
17.50 – 18.10	ANA/MAS-OR05: Veronica Termopoli, Pierangela Palma, Giorgio Famiglini, Maurizio Piergiovanni, Achille Cappiello *Liquid-EI (LEI) Atmospheric Pressure Mechanism for the introduction of liquid streams into an unmodified electron ionization source of a mass spectrometer*
18.10 – 18.30	ANA/MAS-OR06: Chiara Salvitti, Andreina Ricci, Federico Pepi, Stefania Garzoli, Anna Troiani, Giulia De Petris, Marzio Rosi *Selective gas-phase conversion of D-fructose to 5-hydroxymethylfuraldehyde through a base-assisted dehydration process*

Martedi' 12 Settembre 2017

	Sale Paestum A
	*Sessione Parallela: **Sensori***
	*Chair: **Danila Moscone***
9.00 – 9.30	ANA-KN05: Giannetto Marco *Nanobiocomposite-based immunosensors for clinically significant biomarkers*
9.30 – 9.50	ANA-OR18: Maria Pesavento, Nunzio Cennamo, Luigi Zeni, Simone Marchetti *Development of a new platform for chemical sensors based on surface plasmon resonance and molecularly imprinted polymers as receptors*
9.50 – 10.10	ANA-OR19: Patrizia Romana Mussini, Serena Arnaboldi, Ivo Franco Buzzi, Mariacristina La Bianca, Gabriella Natale, Silvia Cauteruccio, Emanuela Licandro, Voichita Mihali, Francesco Sannicolò, Simona Rizzo, Armando Gennaro, Abdirisak Ahmed Isse *Enantioselective voltammetry on achiral electrodes: a comparison between inherently chiral additives based on different stereogenic element*
10.10 – 10.30	ANA-OR20: Paolo Bollella, Dmitry M. Hushpulian, Dónal Leech, Massimo Marcaccio, Gabriele Favero, Franco Mazzei, Lo Gorton, Riccarda Antiochia *Hydrogen peroxide sensitive and selective biosensors based on peroxidases from different sources wired by Os-polymer*
10.30 – 11.00	Coffee Break
	*Sessione Parallela: **Metodologie Avanzate per la Bioanalitica***
	*Chair: **Elisa Robotti***
11.00 – 11.30	ANA-KN06: Gosetti Fabio *Target and non-target LC-MS/MS strategies in environment, food and human health fields*
11.30 – 11.50	ANA-OR21: Ilario Losito, Laura Facchini, Tommaso Cataldi, Francesco Palmisano *Seasonal variations of major phospholipids in mussels of sp. Mytilus galloprovincialis: an investigation by hydrophilic interaction liquid chromatography-electrospray ionization Fourier-transform mass spectrometry*
11.50 – 12.10	ANA-OR22: Carlo Crescenzi, Giuliana Grasso, Francesco Iadaresta, Erik Holmgren *Applications of Porous Graphitic Carbon in liquid chromatography – mass spectrometry*
12.10 – 12.30	ANA-OR23: Pasquale Palladino, Maria Grazia Manera, Roberto Rella, Maria Minunni, Simona Scarano *Synthetic receptors for Troponin T detection by SPR transduction for acute myocardial infarction diagnosis*
12.30 - 12.50	ANA-OR24: Catia Contado *Nano- and micro-particles in food and consumer products: the role of the Field Flow Fractionation techniques in their characterization*
12.50 – 13.10	ANA-OR25: Daniela Cecconi, Marcello Manfredi, Jessica Brandi, Emilio Marengo *Evaluation and comparison of gel-based and gel-free approaches for the in-depth exploration of milk rice proteome*
13.00 – 14.00	Intervallo Pranzo

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	Sale Olimpia
	Sessione Parallela: Ambiente
	Chair: Carlo Crescenzi
9.00 – 9.30	ANA-KN07: Marco Grotti, Andrea Bazzano, Francisco Ardini, Kris Latruwe, Frank Vanhaecke *New insight into the Pb and Sr isotopic compositions of environmental samples from polar regions via single- and multi-collector inductively coupled plasma mass spectrometry*
9.30 – 9.50	ANA-OR26: Francesco Iadaresta, Michele Manniello, Conny Östman, Carlo Crescenzi, Jan Holmbäck, Paola Russo *Aromatic Amines in Textile as Potential Threat for Human Health*
9.50 – 10.10	ANA-OR27: Attilio Naccarato, Antonella Tassone, Sacha Moretti, Rosangela Elliani, Francesca Sprovieri, Nicola Pirrone, Antonio Tagarelli *A greener approach for organophosphate flame retardant determination in airborne particulate matter: microwave-assisted extraction using hydroalcoholic mixture coupled with solid phase microextraction gas chromatography tandem mass spectrometry*
10.10 – 10.30	ANA-OR28: Ivan Notardonato, Mario Vincenzo Russo, Pasquale Avino *Ultrasound-Vortex-Assisted Liquid-Liquid Micro-Extraction for Simultaneous Determination of Organophosphorus Pesticides and Phthalates in Baby Foods*
10.30 – 11.00	Coffee Break

	Sessione Parallela: Ambiente
	Chair: Gianpiero Adami
11.00 – 11.20	ANA-OR29: Marina Di Carro, Barbara Benedetti, Cristiana Mirasole, Emanuele Magi *Calibration of commercial and modified Polar Organic Chemical Integrative Samplers (POCIS) for the analysis of emerging pollutants in waters*
11.20 – 11.40	ANA-OR30: Pascale Raffaella, Bianco Giuliana, Caniani Donatella, Masi Salvatore, Buchicchio Alessandro, Caivano Marianna, Ignazio M. Mancini, Mazzone Giuseppina, Tecla Marinelli *A new validated gas chromatographic-barrier ionization discharge detector (GC-BID) method for the monitoring of CO_2 and N_2O emissions from settling and disinfection units in wastewater treatment plants (WWTPs)*
11.40 – 12.00	ANA-OR31: Sandro Recchia, Carla Tiraboschi, Simone Tumiati, Carlo Dossi, Stefano Poli *Development of a prototype analytical apparatus for the quantitative determination of the composition of fluids in synthetic rocks*
12.00 – 12.20	ANA-OR32: Andrea Speltini, Francesca Merlo, Matteo Contini, Federica Maraschi, Michela Sturini, Lorenzo Malavasi, Antonella Profumo *Solid-phase extraction of trace glucocorticoids from environmental waters on silica-supported humic acids-derived carbons followed by HPLC-ESI-MS*
12.20 – 12.40	ANA-OR33: Alessandro Tinti, Lorenza, Corbella, Cristina Colombi, Eleonora Cuccia, Vorne Gianelle, Satoshi Takahama, Stefania Gilardoni, Paola Fermo *Aliphatic Amines In Atmospheric Aerosol: Sources And Distribution*
12.40 – 13.00	ANA-OR34: Rita Traversi, Silvia Becagli, Laura Caiazzo, Massimo Chiari, Giulia Calzolai, Fabio Giardi, Franco Lucarelli, Angelo Lupi, Mauro Mazzola, Boyan Petkov, Mirko Severi, Vito Vitale, Roberto Udisti *Elemental and Organic Carbon contribution to atmospheric aerosol at Ny Ålesund (Svalbard Islands)*

13.00 – 14.00	Intervallo Pranzo
	Sala Paestum B
14.00 – 15.00	*Sessione Poster 2: Scienza delle separzioni, Bioanalitica, Spettrometria di massa* (ANA PO41-PO85)

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Mercoledi' 13 Settembre 2017

	Sala Paestum B
14.00 – 15.00	*Sessione Poster 3: Elettrochimica-sensori, Spettroscopia, Chemiometria, Forense, poster "ultimo minuto" (ANA PO86-PO140)*
	Sale Paestum A
	Conferenza Plenaria
	*Chair: **Giuseppe Palleschi***
15.00 – 15.45	ANA-PL02: Francesco Ricci *DNA-based nanodevices for clinical application*

	*Sessione Parallela: **Sensori-Elettrochimica***
	*Chair: **Giuseppe Palleschi***
15.50 – 16.10	*Finalisti premio Levi* ANA-IN03: Alessia Amodio, Abimbola Feyisara Adedeji, Matteo Castronovo, Elisa Franco, Francesco Ricci *pH-controlled assembly of DNA tiles*
16.10 – 16.30	ANA-OR35: Alessandro Porchetta, Marianna Rossetti, Giuseppe Palleschi, Francesco Ricci *Co-localization of DNA-based conformational switches for Single-Step Fluorescence Detection of Antibodies*
16.30 – 17.00	Coffee Break
	Sessione Parallela: Sensori-Elettrochimica
	*Chair: **Giovanna Marrazza***
17.00 – 17.30	ANA-KN08: Simona Ranallo, Marianna Rossetti, Andrea Idili, Kevin W. Plaxco, Alexis Vallée-Bélisle, Giuseppe Palleschi, Francesco Ricci *Antibody powered DNA-based nanomachines*
17.30 – 17.50	ANA-OR36: Ilaria Palchetti, Francesca Bettazzi, Luigi Falciola, Valentina Pifferi, Anna Testolin, Chiara Ingrosso, Michela Corricelli *Colloidal Nanoparticle modified Graphene-based electrochemical platforms for clinical biomarker detection*
17.50 – 18.10	ANA-OR37: Donato Calabria, Cristiana Caliceti, Martina Zangheri, Mara Mirasoli, Patrizia Simoni, Aldo Roda *Smartphone–based enzymatic biosensor for rapid detection of oxidase substrates using confined multilayer paper reflectometry*
18.10 – 18.30	ANA-OR38: Stefano Cinti, Luca Fiore, Renato Massoud, Claudio Cortese, Danila Moscone, Giuseppe Palleschi, Fabiana Arduini *A reagentless paper-based screen-printed device to detect chloride ions in serum and sweat*
18.30 – 18.50	ANA-OR39: Donatella Coviello, Michela Contursi, Innocenzo Giuseppe Casella *PVA/GO/Au: development and characterization of a new electrode material*
8.50 – 19.10	ANA-OR40: Paolo Bollella, Daniela A. Stevar, Cristina Tortolini, Lo Gorton, Roland Ludwig, Harry Boer, Anu Koivula, Gabriele Favero, Riccarda Antiochia, Franco Mazzei *Glucose/Oxygen Enzymatic Fuel Cell based on a "green" Gold Nanoparticles modified Graphene Screen-printed Electrode for Glucose detection in human saliva*
19.10 – 19.30	ANA-OR41: Daniele Zappi, Serena Gabriele, Marta Letizia Antonelli, Claudia Sadun *Generation IV ionic liquids and nanomaterials to develop innovative electrochemical biosensing platforms*

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	Sala Olimpia
	Sessione Parallela: ***Scienza delle Separazioni***
	*Chair: **Achille Cappiello***
15.50 – 16.10	ANA-OR42: Riccardo Zenezini Chiozzi, Francesca Ferraris, Carmela Maria Montone, Susy Piovesana, Aldo Laganà *Identification of bioactive peptides derived from cauliflower by-products by multidimensional liquid chromatography and bioinformatics*
16.10 – 16.30	ANA-OR43: Simone Marchetti, Maria Pesavento, Rachele Maria Adele Bernini, Domingo Pastran, Daniele Merli, Letizie DeMaria *Solid phase extraction of dibenzyl disulphide on a molecularly imprinted polymer*
16.30 – 17.00	Coffee Break
	Sessione Parallela: ***Spettroscopia - Chemiometria***
	*Chair: **Giuseppe Spoto***
17.00 – 17.30	ANA-KN09: Ángela I. López-Lorente, Angelo Tricase, Rosaria Anna Picca, Cinzia Di Franco, Maria Chiara Sportelli, Boris Mizaikoff, Christine Kranz, Antonio Valentini, Gerardo Palazzo, Nicola Cioffi *What's new and good in the aqueous synthesis of colloidal gold nanoparticles (AuNPs). A critical overview of AuNPs synthetic methods and their analytical applications*
17.30 – 17.50	ANA-OR44: Maria Chiara Sportelli, Annalisa Volpe, Margherita Izzi, Maurizio Clemente, Rosaria Anna Picca, Amalia Conte, Matteo Alessandro Del Nobile, Antonio Ancona, Nicola Cioffi *Analytical characterization of new composites for food packaging applications*
17.50 – 18.10	ANA-OR45: Marco Minella, Claudio Minero, Matteo Baudino *Photocatalytic transformation of C2Cl4 in gas phase under UV-irradiated TiO2*
18.10 – 18.30	ANA-OR46: Massimo Innocenti, Francesco Di Benedetto, Emanuele Salvietti, Andrea Giaccherini, Walter Giurlani, Francesco Carlà, Nicola Cioffi, Rosaria A. Picca, Enrico Berretti, Roberto Felice *Microscopic and Spectroscopic Analysis with Synchrotron Light of Modified Surfaces of Technological Interest*
18.30 – 18.50	ANA-OR47: Alessandra Biancolillo, Remo Bucci, Andrea Magrì, Federico Marini *Metodi multi-block per l'analisi degli alimenti*
18.50 – 19.10	ANA-OR48: Cristina Malegori, Paolo Oliveri, Maria Alessandra Boggiani, Graziella Pastorini, Monica Casale *An innovative NIR hyperspectral imaging approach to pattern cheese ripening*
19.10 – 19.30	ANA-OR49: Elisa Robotti, Marcello Manfredi, Elettra Barberis, Emilio Marengo *Development of a method for the quality control of polystyrene (EPS) based on the use of microscopy techniques and multivariate statistical methods*

	Sala Athena
*Sessione Congiunta: **Chimica analitica – Chimica dell'Ambiente e dei Beni Culturali***
*Chair: **Fabrizio Passarini***
15.00 – 15.25	ABC/ANA-KN01: Marco Pesce, Andrea Critto, Silvia Torresana, Monia Santini, Elisa Giubilato, Lisa Pizzol, Paola Mercogliano, Alberto Zirino, Ouyang Wei, Antonio Marcomini *An integrated modelling approach to study the impacts of nutrients on coastal aquatic ecosystems in the context of climate change*
15.25 – 15.50	ABC/ANA-KN02: Davide Vione, Birgit Koehler, Francesco Barsotti, Marco Minella, Claudio Minero, Lars J. Tranvik, Tomas Landelius *Photoinduced Processes in Surface Waters: A Photochemical Mapping of Sweden*
15.50 – 16.10	ABC/ANA-OR01: Luca Carena, Silvia Berto, Davide Vione, Federico Valmacco, Matteo Marletti, Agnese Giacomino, Claudia Barolo, Roberto Buscaino, Mery Malandrino *Application of an electro-activated glassy carbon electrode (GCE) to the analytical monitoring and photochemical studying of acetaminophen.*
16.10 – 16.30	ABC/ANA-OR02: Luca Rivoira, Alessandra Lo Re, Davide Vione, Marco Minella, Marta Appendini, Barbara Onida, Maria Concetta Bruzzoniti *Removal and photodegradation of glyphosate herbicide from waters using a mesoporous substrate with adsorbent properties*
16.30 – 17.00	Coffee Break
*Sessione Congiunta: **Chimica analitica – Chimica dell'Ambiente e dei Beni Culturali***
*Chair: **Andrea Gambaro***
17.00 – 17:30	ABC/ANA-KN03: Alessandra Genga, Maria Siciliano, Cosimino Malitesta, Tiziana Siciliano, Antonio Proto, Raffaele Cucciniello *Primary and secondary carbonaceous species in PM10 and PM2.5 samples*
17.30 – 17.50	ABC/ANA-OR03: Sara Padoan, Elena Barbaro, Matteo Feltracco, Roberta Zangrando, Carlo Barbante, Andrea Gambaro *Characterization of ionic composition in the submicron fraction of urban aerosol*
18.10 – 18.30	ABC/ANA-OR04: Mirko Severi, Silvia Becagli, Laura Caiazzo, Virginia Ciardini, Ester Colizza, Fabio Giardi, Karin Mezgec, Claudio Scarchilli, Barbara Stenni, Elizabeth R. Thomas, Rita Traversi, Roberto Udisti *Sea salt sodium record from Talos Dome (East Antarctica) as a potential proxy of the Antarctic past sea ice extent*
18.30 – 18.50	ABC/ANA-OR05: Roberto Udisti, Silvia Becagli, Ezio Bolzacchini, Laura Caiazzo, David Cappelletti, Luca Ferrero, Fabio Giardi, Marco Grotti, Angelo Lupi, Mery Malandrino, Mauro Mazzola, Beatrice Moroni, Mirko Severi, Rita Traversi, Vito Vitale *A six-year record of size distribution and chemical composition of Arctic aerosol. Main results and future bi-polar perspetives*
18.50 – 19.10	ABC/ANA-OR06: Janeth Tafur Marinos, Marco Ginepro, Vincenzo Zelano *Effects of biomass, temperature and thermochemical process on PAH concentration in biochar*
19.10 – 19.30	ABC/ANA-OR07: Ezio Bolzacchini, Luca Ferrero, Antonio Proto, Concetta Pironti, Raffaele Cucciniello, Oriana Motta, Cristiana Rizzi, Grisa Močnik, Lorenza Dall'Aglio, Chiara Rostagno *Particulate and gaseous indoor contamination at the Museum of "Last Supper" of Leonarndo Da Vinci: results from one year of monitoring activity*

Medaglie e Premi
della Divisione di Chimica Analitica

Medaglia "*Arnaldo Liberti*" - 2017

Prof. Aldo Laganà, *Università degli Studi di Roma "La Sapienza"*

Medaglia "*Canneri*" - 2017

Prof. ssa Luigia Sabbatini, *Università degli Studi di Bari "Aldo Moro"*

Premi alla Ricerca

"Premio Giovane Ricercatore" **- 2017**

Dott. ssa Susy Piovesana, *Università degli Studi di Roma "La Sapienza"*

"Premio di Laurea" **- 2017**

Dott. ssa Carmela Maria Montone, *Università degli Studi di Roma "La Sapienza"*

Conferenze Plenarie

- [ANA PL01](): Luisa Torsi, *Università degli Studi di Bari "Aldo Moro"*
- [ANA PL02](): Francesco Ricci, *Università degli Studi di Roma "Tor Vergata"*

Printable Organic Bioelectronics for Bioanalytical Detections at The Physical Limit: Is This Feasible?

Luisa Torsi[a]

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Counting the molecules present in a solution instead of assaying its concentration is the ultimate and visionary goal in chemical analysis. It's actuation however, necessarily requires reliable technologies that can "count" the molecules one by one. Such an approach would enable not only fundamental understanding of subtle effects in an affinity interaction likely hidden in ensemble measurements, but also it would pave the way to striking applications. Indeed, proteins and biomolecules detection at the physical limit are foreseen to generate ground-breaking technological fallouts such as for instance, label-free biosensors endowed with high selectivity as well as sub attomolar (10^{-18} M, aM) sensitivity for non-invasive label-free quantitative analysis of pathogens or diseases' markers in bio-fluids such as saliva or tears.

Interestingly, cells can track single molecules by decoding the response mediated by millions of highly packed receptors residing on their surface. By this mean, sperm cells sense few chemoattractants to localize the oocyte while rod cells on the retina respond to a single photon. Conversely, label-free single-molecule detection has been achieved so far by funnelling a large number of ligands into a sequence of single binding events through few recognition elements host on a nano-sized transducing interface. For instance, single DNA detection has been demonstrated using few bio-probes attached to a single nanotube transistor. An approach involving nano-devices is therefore inherently unable to track few cues in a milieu as either very high ligand concentrations or impractical long times are required. Conceptualizing cells' ability to track recognition events at the physical limit, the use of an electrolyte gated organic field-effect transistor bio-functionalized with highly packed antibodies is presented and the selective and reliable electronic detection of an extremely low concentration of affinity ligands is demonstrated.[1-5]

The present lecture thus aims at presenting an overview on the challenges and on the exciting perspectives for Analytical Chemistry and Chemistry in general, that are associated with the quantification of proteins down to few molecules. An outlook on the extremely high-performance level of millimetre-size organic bioelectronic sensors integrating a trillion of capturing molecules, will be provided showing that sub-aM detection limits can be reliably reached.[1] As cases of studies, the selective and ultra-sensitive assay of immunoglobulins and C-reactive protein in saliva will be discussed. The organic bioelectronic transistors used are mm-size, low-cost and are operated at physiologically relevant conditions as well as in human saliva setting the ground for a revolution in immunoassay for early bio-markers detection.

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DNA-Based Nanodevices For Clinical Applications

Francesco Ricci[a]

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DNA nanotechnology uses synthetic DNA (or nucleic acids) as a versatile material to rationally engineer nanoscale tools and molecular devices.

During this presentation, I will give an overview of the most representative and recent examples developed in our lab in this research field. More specifically, I will show how to exploit the designability of DNA to fabricate nature-inspired DNA-based nanodevices that are designed to undergo a conformational change (switch) upon binding to a specific input (pH[1-4], antibodies[5-6], enzymes[7], small molecules[8-10], etc.). This input-triggered conformational change can be used for diagnostic, drug-delivery or synthetic-biology applications.

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Keynote e Conferenze su invito

- [ANA KN 01](): Sara Bogialli, *Università degli Studi di Padova.*

- [ANA KN 02](): Martina Zangheri, *Università degli Studi di Bologna "Alma Mater Studiorum".*

- [ANA/ELE/GS KN 01](): Fabiana Arduini, *Università degli Studi di Roma "Tor Vergata".*

- [ABC/ANA/FIS KN 01](): Rocco Mazzeo, *Università degli Studi di Bologna "Alma Mater Studiorum".*

- [ABC/ANA/FIS KN 02](): Erika Ribechini, *Università degli Studi di Pisa.*

- [ANA/ELE/GS KN02](): Andreas Lesch, *École Polytechnique Fédérale de Lausanne (EPFL) Valais, Svizzera.*

- [ANA KN 03](): Silvia Becagli, *Università degli Studi di Firenze.*

- [ANA KN 04](): Paola Donato, *Università degli Studi di Messina.*

- [ANA/MAS KN01](): Cosima Damiana Calvano, *Università degli Studi di Bari "Aldo Moro".*

- [ANA/MAS KN02](): Danilo Sciarrone, *Università degli Studi di Messina.*

- [ANA KN 05](): Marco Giannetto, *Università degli Studi di Parma.*

- [ANA KN 06](): Fabio Gosetti, *Università degli Studi del Piemonte Orientale.*

- [ANA KN 07](): Marco Grotti, *Università degli Studi di Genova.*

- [ANA KN 08](): Simona Ranallo, *Università degli Studi di Roma "Tor Vergata".*

- [ANA KN 09](): Nicola Cioffi, *Università degli Studi di Bari "Aldo Moro".*

- [ABC/ANA KN01](): Antonio Marcomini, *Università degli Studi di Venezia "Ca' Foscari".*

- [ABC/ANA KN02](): Davide Vione, *Università degli Studi di Torino.*

- [ABC/ANA KN03](): Alessandra Genga, *Università del Salento.*

- [ANA IN01](): Susy Piovesana, *Università degli Studi di Roma "La Sapienza".*

- [ANA IN02](): Anna Laura Capriotti, *Università degli Studi di Roma "La Sapienza".*

- [ANA IN03](): Alessia Amodio, *Università degli Studi di Roma "Tor Vergata.*

High Resolution Mass Spectrometry: The Re-Evolution Of The Risk Assessment And Management

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The evolution and the spreading of high resolution mass spectrometry (HR-MS) coupled to gas or liquid chromatography (GC or LC) has suggested the implementation of new analytical workflows for the detection of unknown compounds. In the last ten years, a huge number of scientific papers based on HR-MS highlighted the presence of natural and synthetic organic substances that are of concerns in the environmental, food, clinical and forensic fields.

Anyway, the policy actions require much more information than those provided by the analytical screening, and the approach based on risk assessment is essential. It is clear that the chemical snapshot of a possible contamination is not exhaustive, and inter-sectorial expertise are necessary to correctly understand the health risks.

Actually, HRMS is still underused in the framework of the official analyses, even because the harmonization of the protocols and the data interpretation remain important drawbacks. Furthermore, the need of quantifying compounds with potential adverse toxicological effect is surely the challenge to be faced out.

Recently, some new trends has become more common among stakeholders involved in the risk management of the environment, such for the water analysis. The adoption of the Water Safety Plans is spurring the integration between the available analytical platforms based on HR-MS and the "decision making" actions.

Some of the main limitations and perspectives related to the revolution of the risk management inspired by the HR-MS is here presented and discussed.

Biosensors Based on Chemiluminescent-Lateral Flow Immunoassay: A Powerful Approach For Integrated Analytical Platforms

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Lateral Flow Immunoassay (LFIA) is a technology currently widely applied in resource-poor or non-laboratory environments (point-of-care, POC) that is based on prefabricated strips of a carrier material containing dry reagents that are activated by applying the fluid sample. The conventional LFIA are available mostly for qualitative analyses, but the use of enzymes as tracers, coupled with chemiluminescence (CL) detection, provides highly sensitive quantitative assays[1]. Biosensors based on CL-LFIA are very promising analytical tools for rapid on-site detection of analytes in complex matrices.

Here, we report about the latest advances in CL-LFIA based technologies and their applications in different fields. Thanks to the intrinsic simplicity of CL detection principle, several portable and sensitive CL detectors can be designed to obtain a fully-integrated system. In addition, to reach the goal of portability, ready-to-use disposable cartridges containing all the reagents necessary to complete the analysis must be developed.

Nowadays, the improved imaging technology of the Back Side Illuminated Complementary Metal Oxide Semiconductor (BI-CMOS) sensors used in smartphone cameras make them suitable for fast and accurate POC diagnosis based on CL-LFIA[2-3]. These biosensors will be useful in all situations where a decentralized and fast detection is required, taking advantage of the location (GPS) and wireless long distance data transfer ability. We applied this technology in different fields, from the detection of clinical biomarkers to the evaluation of mycotoxins food contamination. In particular, different formats for the LFIA cartridges were designed for combining compactness and ease of use. Accessories able to transform the smartphone into a CL detector were developed using a desktop 3D printer.

Another CL detector suitable for on-site applications are thin film hydrogenated amorphous silicon (a-Si:H) photosensors[4] that have recently been proposed as a low-cost and versatile technology for developing a miniaturized biosensor with high detectability and sensitivity. We developed a novel biosensor based on CL-LFIA method with integrated amorphous silicon (a-Si:H) photosensors array to quantitatively detect human serum albumin (HSA) in urine samples. This combination provided compact, sensitive and potentially low-cost microdevices for CL and bioluminescence-based bioassays with a wide range of possible applications for in-field and POC bio-analyses.

Finally, the last challenge is to exploit the simplicity and portability of the CL-LFIA based device for performing salivary biomarker analysis onboard the International Space Station (ISS). A portable biosensor was developed for salivary cortisol quantitative detection, which will be used by crew members for monitoring their health status during space missions.

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New Sustainable and Cost-Effective Paper-Based Electrochemical (Bio)Sensors

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Electronic equipment generated 9 million tons of waste in 2005 in EU, and it is expected to grow to more than 12 million tons by 2020. To decrease the environmental pollution of electronics, the development of sustainable sensors, including paper-based electrochemical devices, are attracting growing interest thanks to their cost-effectiveness, suitability for in situ analysis, and environmentally friendly.

In this keynote, we reported the recent advancements on paper-based electrochemical (bio)sensors for environmental, biomedical, and agrifood sectors carried out by our group.

We described a novel reagentless paper-based electrochemical phosphate sensor, manufactured with a simple and inexpensive approach. By following three easy steps, consisting of wax patterning, paper chemical modification, and electrode screen-printing, the filter paper provides an effective electroanalytical platform to sense phosphate ions in standard solutions and in real samples (river water). This novel and highly sustainable configuration allows for the determination of phosphate ions with high reproducibility thanks to the use of heptamolybdate as reagent loaded on paper and carbon black as ink nanomodifier, achieving a detection limit of 4 mM. The filter paper has been also combined with the butyrylcholinesterase enzyme (BChE) for the detection of pesticides in rivers and wastewaters. The principle of this approach is based on dual electrochemical measurements, in parallel, of butyrylcholinesterase enzyme activity towards butyrylthiocholine, with and without exposure to contaminated samples. The sensitivity of this device is largely improved using a carbon black/Prussian Blue nanocomposite as a working electrode modifier. A strip of a nitrocellulose membrane, that contains the substrate, is integrated with a paper-based test area that holds a screen-printed electrode and BChE, allowing a reagent-free detection of Paraoxon down to 3 μg/L. Beside the filter paper, the office paper has also been exploited as substrate to print the electrode. An office-paper based sensor has been developed for monitoring Zn(II) in biological fluids. The printed sensor modified with bismuth film has been used to detect it by stripping analysis, with a detection limit of 25 ng/mL and a relative standard deviation of 8%. To highlight the feasibility, reliability, and easiness of the proposed electrochemical sensor, Zn(II) has been detected in serum and sweat at a physiological level (μg/mL). The sensor printed on office paper has been also combined with alcohol oxidase enzyme for the detection of ethanol in beer samples. After optimizing the analytical parameters, such as pH, enzyme, concentration, and working potential, the developed biosensor allowed a facile quantification of ethanol up to 10 mM, with a detection limit equal to 0.52 mM. Recently, we have also combined the sensor fabricated using wax printing and screen-printing technologies with a printed holder made by a 3-D printing technology. This device is able to measure the BChE activity in serum, with a linear range up to 12 UI/mL and a detection limit lower than 1 UI/mL.

Macro Mid-Infrared Total Reflection (MA-FTIR) Mapping for the Characterization of Outdoor Bronze Surfaces

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In the last decades, many research efforts have been devoted to the development of advanced non-invasive approaches for the examination of cultural heritage. However, up to now, too few attentions has been paid on the potentialities of the Macro Mid-Infrared total reflection (MA-FTIR) mapping analysis. Indeed, the infrared reflectance spectroscopy single point investigation has been largely applied in analytical studies of artworks due to its advantage of characterizing both organic and inorganic materials. On the other hand, the possibility to simultaneously obtained information on molecular composition and spatial distribution of constituents is of crucial importance for properly addressing conservation issues.

To this aim, the present research work was focused on the application of an advanced MA-FTIR mapping system for the study of altered bronze surfaces. Moreover, an *ad hoc* multivariate approach has been proposed for the interpretation of data.

A bronze sculpture exposed in the atmosphere deteriorates with time, developing a greenish/blackish layer mainly constituted of corrosion products. The formation of these corrosion products implies complex chemical, electrochemical and physical processes that strongly depend on the constituents of the surrounding environment. In addition, different acrylic resins, synthetic waxes and organic inhibitors may be commonly applied for short-term protection of outdoor bronze sculptures.

To the Authors knowledge for the first time Mid FTIR macro mapping analysis was performed on metal patinas, allowing the location of corrosion products and old coatings, and describing their interactions. The performances of the approach have been initially evaluated on standard bronze samples characterized by the presence of a green basic hydroxysulfate (brochantite) treated with different organic materials. Subsequently, the exceptional case of study of the bronze sculptures of the Neptune Fountain (Bologna, 16th century) has been investigated.

The instrument acquired hyperspectral cubes by scanning the metal surface without any contact, recording sequential total reflection single-point spectra. The big dimension of the data cubes obtained, as well as, the deformation of IR bands induced by specular reflection phenomena, may serious hampering the correct interpretation of the spectral features. Thus, a chemometric method, based on the use of the brushing procedure, was proposed to extract all the useful information embedded in a complex hypercube.

The high specificity of MA-FTIR mapping revealed important outcomes on the state of conservation of the monumental statue. In particular, it was possible to clearly describe well preserved areas in which wax and incralac resulted to be still present, describing their distribution and identifying zones affected by a more aggressive corrosion process.

GC/MS and HPLC/MS Characterization of Organic Residues from Ceramic Vessels of Basque Whalers of the 16th to 17th Century

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The aim of this work is focused on the identification of the organic residues from ceramic vessels recovered from a deposit in Lekeitio (Basque Country, Northern Spain). These vessels are suspected to have been used by the Basque whalers in the period from 16th to 17th Century to store whale oil. Bearing in mind our aim, different analytical approaches based on chromatography and mass spectrometry (GC/MS and HPLC-ESI-qTOF) were used to study both fresh blubber and whale oil products and the archaeological substances in order to obtain chromatographic profiles and possibly detect highly diagnostic biomarkers. Two hydrolysis approaches were used in order to compare data and obtain as much information as possible; (i) microwave assisted direct hydrolysis of the ceramic samples and (ii) microwave assisted hydrolysis of previously solvent-extracted samples. On the other side, the study of solvent (chloroform:hexane (3:2 v/v)) soluble fraction was carried out by means of HPLC-ESI-qTOF. Preliminary results led to the identification of some interesting organic compounds in the hydrolysable and soluble aliquots from the ceramic vessel such as pristane, phytane, several saturated fatty acids, hydroxyacids and cholesterol along with triacylglycerols in the solvent soluble fraction. On the other hand, the obtained results for the fresh whale oil and blubber suggested a similar lipid profile to the ones obtained for the archaeological samples. The chemical results together with archaeological data seem to indicate the vessels were used to store whale oil.

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Preparing Nanoparticles on Large Electrodes Using Inkjet Printing and Flash Light Irradiation

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Coating electrodes with catalytically active metal and alloyed metal nanoparticles (NPs) can improve significantly the performance of commercial electrocatalytic and (bio)analytical devices. Nevertheless, the price of many noble and scarce metals requires sustainable strategies to synthesize NPs and to prepare nanoparticle-decorated surfaces. In addition, the cost-effective and high-throughput optimization of the NP properties as a result of the NP size, shape, composition and synergistic effects with support materials is very attractive for academia as well as industry. This is especially true if process conditions are easily tunable, highly reproducible and fully up-scalable.

The fabrication of NP-coated electrode surfaces can be achieved in various ways, usually started with a NP synthesis, with or without support particles and dispersed in solution, before an ink or paste is formulated. Subsequently, this mixture is casted by dropping, dispensing or printing onto the sensing device structure. Afterwards, thermal post-processing results in the removal of solvents and stabilizers achieving electrodes with catalytically active and well-adhered NPs. Alternative strategies apply metal precursors that are electrochemically, (photo)chemically or thermally (thermal decomposition by equilibrium heating in a furnace) converted into the according NPs.

In this contribution, we demonstrate our novel approach where defined thin films of metal precursors, e.g. chloroplatinic acid, are first deposited onto various electrode surfaces by using inkjet printing and then irradiated with a high energy light pulse from a Xenon flash lamp to generate NPs within a fraction of a second[1]. Due to the rapid absorption of the flash light by the precursor-coated electrodes temperatures of several hundred degrees Celsius are generated immediately inside the printed films. The result is a rapid thermal decomposition of the precursor into the pure metal or alloyed metal nanoparticles. As for the well-known thermal decomposition processes at equilibrium heating conditions, side products are simply gaseous (e.g. HCl, Cl_2 and H_2O) leaving pure metal on the electrode surface[2,3].

We use an integrated inkjet printing (with three printheads) and flash light irradiation platform to prepare the NP-decorated electrodes (e.g. Pt deposited on ITO, graphene or carbon nanotubes) of square centimeter size only within few minutes at ambient conditions[4]. This allows a rapid optimization and up-scaling of the process outcome. The final electrodes were optically and electrochemically analyzed, the latter in particular with Soft-Probe Scanning ElectroChemical Microscopy (Soft-Probe-SECM)[5].

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Multidisciplinary Approach to Constrain the Ship Contribution to the Aerosol of the Central Mediterranean Sea

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Ship emissions may significantly affect atmospheric concentrations of several important pollutants, especially in maritime and coastal areas[1]. In spite of the great amount of gas and particulate arising from ship emission, maritime transport is relatively clean if calculated per kilogram of transported material, and it is currently increasing with respect to air and road transport[2]. In addition, emissions from other transport sectors are decreasing due to the implementation of advanced emission reduction technologies, and the relative impact of shipping emissions is increasing. PM_{10} aerosol samples were collected during summer 2013 within the framework of the Chemistry and Aerosol Mediterranean Experiment (ChArMEx) at two sites located North (Capo Granitola, 36.6°N, 12.6°E) and South (Lampedusa Island, 35.5°N, 12.6°E), respectively, of the main Mediterranean shipping route in the Sicily Channel. The PM_{10} samples were collected with 12 hours time resolution at both sites. Selected metals, main anions, cations, and elemental and organic carbon were determined. The evolution of soluble V and Ni concentrations (typical markers of heavy fuel oil combustion) was related to meteorology and ship traffic intensity in the Sicily Channel, using a high resolution regional model for back trajectories calculation. Elevated concentration of V and Ni were associated with transport from the Sicily Channel and coincidences between trajectories and positions of large ships, both at Capo Granitola and Lampedusa; the vertical structure of the planetary boundary layer also appears to play a role, with high V values associated with strong inversions and stable boundary layer. The V concentration was generally lower at Lampedusa than at Capo Granitola, where it reached a peak value of 40 ng/m^3. Concentrations of rare earth elements, La and Ce in particular, were used to identify possible contributions from refineries, whose emissions are also characterized by elevated V and Ni amounts; refinery emissions are expected to display high La/Ce and La/V ratios, due to the use of La in the fluid catalytic converter systems. In general, low La/Ce and La/V ratios were observed in the PM samples, allowing to unambiguously identify the large role of the ship source in the Sicily Channel. Based on the sampled aerosols, ratios of the main aerosol species arising from ship emission with respect to V were estimated with the aim of deriving a lower limit for the total ship contribution to PM_{10}. The estimated minimum ship emission contributions to PM_{10} was 1.9 μg/m^3 at Lampedusa, and 2.8 μg/m^3 at Capo Granitola, corresponding to 11% and 8.2% of PM_{10}, respectively.

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Supercritical Fluid Chromatography × Reversed-Phase Liquid Chromatography with Quadrupole Time-Of-Flight and Ion Mobility Mass Spectrometry Detection: a Proof of Concept

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On line comprehensive liquid chromatography LC×LC techniques are becoming increasingly popular when dealing with separation of complex mixtures of non-volatile components. The maximum gain in separation power arises from the combination of independent separation mechanisms (stationary phases) and, to this regard, the combination of normal phase (NP) and reversed phase (RP) represents one of the most effective ways to increase orthogonality. However such a coupling poses important technical challenges, mainly related to the immiscibility of the mobile phases, peak focusing at the head of the secondary column (^{2}D), and signal interferences. On the other hand, seemingly compatible systems, such as RP-LC×RP-LC, suffer from high correlation of the separation, and peak deterioration due to viscous fingering effects.

Supercritical fluid chromatography (SFC) may be a valuable alternative to NP-LC in the first separation (^{1}D) dimension, delivering complementary selectivity while alleviating immiscibility issues and further providing a number of advantages related to the use of supercritical CO_2.

Here the development of a fully automated SFC × RP-LC instrument is described, configured around two 2-position, six-port high pressure switching valves equipped with two packed octadecyl silica cartridges for effective trapping and focusing of the analytes after elution from ^{1}D. After depressurization of the effluent by the backpressure regulator (BPR), a water make-up flow was added prior to entering the loops, to efficiently focus the solutes on the sorbent material and reduce interferences of expanded CO_2 gas on the second dimension separation.

In addition to PDA and QToF-MS detection, ion mobility separation based on analyte mass, shape and size added a fourth separation dimension for a comprehensive characterization of the sample. With such a system, carotenoids fingerprinting by in a paprika sample was obtained in half the time with respect to conventional NP-LC×RP-LC, with superior resolution in ^{2}D and extended ^{2}D column lifetime. The separation of geometric isomers like cis/trans carotenoids differing in collision cross-sections was demonstrated, using travelling wave ion mobility.

Advanced Mass Spectrometric Techniques for the Untargeted Lipidome Characterization of Fibroblasts in Early On-Set Parkinson's Disease Patients

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Parkinson's disease (PD) is a progressive neurodegenerative disease involving the nigrostriatal pathway, where patients manifest dysfunction in motor symptoms when more than 50% of neurons are lost [1]. Although it is well recognized that alterations of lipid signaling and metabolism plays a significant role in many human diseases [2], little is known about the role of lipids associated with this specific disease. Recently, it has been reported that altered lipid pathways in the primary visual cortex and the anterior cingulate are possible in neurological disorders such as PD by analyzing post-mortem tissues from patients in advanced neuronal degeneration stage [3]. Such an approach, however, hinders the identification of the first neuronal changes. Thus, understanding the mechanisms of PD and identifying neuronal changes in the early phase of PD, by recurring to samples alternative to post-mortem biopsies, represents an urgent and challenging task.

According to their polygenic predisposition and environmental etiopathology [4], skin fibroblasts are now widely recognized as a useful model of primary human cells, capable of reflecting the chronological and biological aging of the patients. A lipidomics study of easily accessible primary human fibroblasts is presented here based on hydrophilic interaction liquid chromatography coupled to electrospray ionization-Fourier transform mass spectrometry, using both positive and negative polarities [5]. After testing different extraction protocols, the Bligh-Dyer method was shown to provide the largest number of recovered lipids. Thus, phospholipids (PL) from dermal fibroblasts of two unrelated PD patients with different parkin mutations and two controls were characterized by recurring to single and tandem MS measurements on a hybrid quadrupole-Orbitrap mass spectrometer. This untargeted approach enabled the identification of various PL classes as phosphatidylcholines (PC), phosphatidylethanolamines (PE), lysoPC, lysoPE, phosphatidylinositols, phosphatidylserines, sphingomyelins, mono-, di- and tri-hexosylceramides and ganglioside GM1, GM2 and GM3. To identify the main lipids and/or lipid classes involved in the pathological condition of PD, lipidomics data on a higher number of samples need to be collected and processed by multivariate statistical analyses. In this communication, an interesting set of preliminary findings will be reported and discussed.

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Advanced Analytical Capabilities Exploiting Isotope Ratio Mass Spectrometry and Quadrupole Mass Spectrometry Coupled to Multidimensional Gas Chromatography

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Isotope Ratio Mass Spectrometry (IRMS) is commonly recognized to be able to provide information about the geographical, chemical, and biological origins of substances. The ability to determine the source of substances stems from the relative isotopic abundances of the elements which comprise the material. By performing a separation prior to isotope ratio analysis, hyphenated techniques such as GC-C-IRMS, can provide isotopic analysis of a complex mixture, thereby providing additional information and higher discriminatory power. Since its introduction, the use of this analytical approach was not widespread due to a series of drawbacks related to chromatographic and isotopic issues. In fact, dead volumes due to the typical instrumental setup, requiring the combustion of the components followed by a drying step, often limit the separation efficiency, driving to an increased band broadening and peak asymmetry producing peak coelutions, thus falsify the measurements. Moreover, the reduced chromatographic performance increases the gas chromatographic isotope effect (or inverse isotopic effect) that generates GC peak not isotopically consistent because composed of lighter isotopes (^{12}C, ^{1}H and ^{16}O) that elute after the isotopomers containing heavier organic compounds because of their higher volatility. The present research deals with the development of an MDGC-MS/IRMS prototype characterized by the improved resolution capability of the heart-cut mode, exploiting two different GC stationary phases, and the simultaneous qMS and IRMS detection of the 2D chromatographic bands. The IRMS system was optimized in terms of dead volumes enabling to overcome the extra-column band broadening effect that usually affects the commercial systems. Different applications on food and flavour and fragrance samples are reported showing the enhanced performances of the prototype described.

Nanobiocomposite-Based Immunosensors for Clinically Significant Biomarkers

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An outlook on the diagnostic applications of nanobiocomposite-based immunosensors implemented on the working principle of competitive or noncompetitive ELISA assays is given with an emphasis on applications in life sciences. Among biosensors, electrochemical immunosensors are very powerful and versatile tools for rapid and user-friendly screening analysis of biomarkers of clinical concern[1], thanks to their compatibility with portable and compact instrumentation, exploiting disposable screen-printed electrodes as sensing substrates[2]. These devices match the fundamental needs required for the screening analysis of biological samples in terms of reliability, simplicity of operation and of the analytical platform, fast response time and cost-effectiveness, associated to good sensitivity and specificity assured by targeted antibodies.
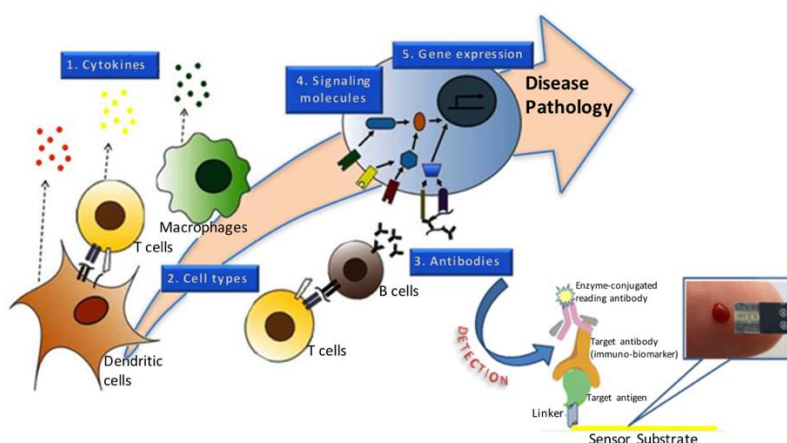
Enhanced analytical performance required for diagnostic purposes can be achieved exploiting the peculiar features of atomic or molecular nanomaterials, such as gold nanoparticles, carbon nanotubes and dendrimers, properly conjugated with the target bioreceptors. Nanobiocomposite-based immunosensors exhibit improved sensitivity thanks to their high active surface area, associated to the enhancement of the electron transfer in the diffusive phenomena involved in the transduction mechanisms. In addition, due to the absence of significant matrix effect, these devices are particularly suitable for direct analysis of physiological fluids, with excellent LODs achievable under careful optimization of the operating conditions. In this context, all the developed devices here described were optimized by multivariate experimental design approach and fully validated in different physiological fluids for determination of protein biomarkers (antigens, antibodies or enzymes) specifically related to oncological, autoimmune or infective diseases[3-8].

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Target and Non-Target LC-MS/MS Strategies in Environment, Food and Human Health Fields

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In the last years, modern LC-MS/MS technique have experienced an impressive progress, both in terms of technology development and application.

Identification of unknown analytes in complex samples has always represented one of the challenge for analytical chemistry. LC-MS/MS techniques can give an answer, supporting the so-called non-target analysis, a recent analytical strategy, which aims to give the comprehensive identification and quantification of the chemical species of interest in complex samples.

The versatility of LC-MS/MS technique not only permits the development of sensitive, precise and accurate methods for the identification and quantification of target species, but also the possibility of searching for non-target compounds without having any *a priori* knowledge about them.

The increasing use of hybrid MS analyzers and in particular high-resolution MS ones allowed the growth of non-target strategies, not only by using the traditional data dependent mode, but also through the data independent acquisitions.

Water-pollutant monitoring makes typically use of methods developed for target analysis, focused on priority pollutants. However, the monitoring of target-compounds is often insufficient to definitely assess the quality of surface water, because the presence of only a limited number of potential pollutants is considered, whereas the simultaneous presence of potentially harmful non-target pollutants must be taken into account. For example, pollutants can undergo degradation for action of sunlight, temperature, and bacteria, forming transformation products often more toxic than their precursors. The degradation studies of some pesticides in paddy water permitted to develop simultaneously target and non-target methods through the data independent acquisition mode.

In the field of food analysis, it is possible to investigate the modifications taking place in food and beverages to identify toxic or hazardous species that may form, because of reactions occurring among different ingredients, transformation reactions, environmental effects or releases from packaging materials. These potential interactions take generally place in post-production phases and during the pre-selling storage and conservation. The interactions are often unpredictable and can give rise to different types of contamination with the formation of new species potentially harmful to the health, For example, the photoirradiation of the beverages containing food dyes can cause the formation of degradation products due to interactions with the other components of the beverage itself. The elucidation of the degradation product chemical structures has been proposed on the basis of HR MS/MS acquired data.

Recent studies in human health field concern the topic of premature newborns to investigate the possible correlation with the presence and accumulation in newborn plasma of endocrine disrupting compounds (EDCs). Premature infants need intensive medical care and they must be nourished only with parenteral solutions by different medical devices for almost 2-3 months. For this purpose, a high throughput online SPE UHPLC-MS/MS method has been developed and allowed the determination of 21 EDCs in plasma samples of premature newborns and in the physiological solutions used for leaching experiments from these medical devices.

New Insight into the Pb and Sr Isotopic Compositions of Environmental Samples from Polar Regions via Single- and Multi-Collector Inductively Coupled Plasma Mass Spectrometry

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Measurement of isotope ratios is considered a hot topic for both the analytical and geochemical scientific communities because of the unique information that they can reveal. In particular, in the context of environmental studies, Pb and Sr isotope ratios can help to discriminate between natural and anthropogenic inputs, as well as to identify their source regions and transport pathways.

Pb shows pronounced variation in its isotopic composition because three out of its four isotopes, ^{206}Pb, ^{207}Pb and ^{208}Pb, are the stable end products of the decay chains of ^{238}U, ^{235}U and ^{232}Th, respectively. As a consequence, there are relatively large differences in the $^{208}Pb/^{207}Pb$ and $^{206}Pb/^{207}Pb$ values for crustal and ore Pb deriving from various locations and mining areas. Contrary to Pb, Sr is typically of natural origin; however, since ^{87}Sr is produced via the β^--decay of ^{87}Rb, natural variations of $^{87}Sr/^{86}Sr$ in rocks occur, and the determination of this isotope ratio can be used for tracing the geographical source(s) of mineral dust.

The accurate measurement of Pb and Sr isotope ratios in environmental samples collected from polar regions can, however, be quite challenging, due to the low analytical concentration, matrix effects, risk of contamination and the limited sample size.

In recent years, we have developed new analytical procedures based on both single- and multi-collector inductively coupled plasma mass spectrometry, able to provide fit-for-purpose performances for a number of environmental matrices, including marine suspended particulate matter and sediment, atmospheric particulate and surface snow. The methods were fully 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).

The main features of the analytical procedures developed and representative results concerning the marine environment and the atmosphere will be presented and discussed.

Antibody powered DNA-based nanomachines

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Taking inspiration from naturally-occurring receptors, which often signal the presence of their molecular target via binding-induced conformational changes, we developed two different DNA-based nanomachines that are activated by specific antibodies. More specifically, in the first case we designed a conformational switching, optically signaling stem-loop DNA nanodevice that supports the introduction of two copies of a wide range of polypeptide, small molecule, or oligonucleotide recognition elements (1). The binding of the antibody to the DNA nanomachine causes a structural change, producing a fluorescence signal monotonically related to the target's concentration. The nanomachine can be adapted to the detection of any antibody by simply changing the recognition element. Moreover the nanomachine does not need to be chemically activated, it is rapid (acting within five minutes) enabling the targeted antibodies to be easily detected, even in complex clinical samples such as blood serum and may prove to be useful in a range of different applications such as point-of-care diagnostics and bioimaging.

In the second case, we have designed a new class of DNA based nanomachines that can reversibly load and release a molecular cargo upon the binding of a target antibody. Our strategy to rationally design an antibody-driven DNA-based nanomachine involves the use of a triplex forming DNA strand that is designed to recognize a specific DNA strand through the formation of a clamp-like triplex forming mechanism that involves both Watson-Crick and Hoogsteen interactions and which is conjugated at the two ends with a pair of antigens (2). Antibody binding to the two antigen tags in the nanomachine causes a conformational change that energetically disrupts the triplex-forming Hoogsteen interactions in the triplex complex thus destabilizing the nanomachine/cargo complex. The design principle for antibody-powered release of a molecular cargo strand is highly generalizable and can easily be adapted to other antibodies via the expedient of changing the recognition element employed. We have demonstrated here that our approach can be extended to different triggering antibodies and the effect can be specific and selective enough even in complex media (90% serum).

Given these attributes, the antibody-poweered DNA nanomachines we have developed here may prove of utility in a range of applications, including point-of-care diagnostics, controlled drug-release and in-vivo imaging.

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What's New and Good in the Aqueous Synthesis of Colloidal Gold Nanoparticles (AuNPs). A critical Overview of AuNPs Synthetic Methods and their Analytical Applications.

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Gold nanoparticles (AuNPs) are receiving increasing attention in several fields, such as biomedicine[1], sensors[2] and nanoparticle-assisted spectroscopies[3] and spectrometry[4], just to cite a few. Among the methods for AuNPs preparation, the chemical reduction of gold(III) species is widely applied, based on the use of tetrachloroauric acid ($HAuCl_4$) as precursor and sodium citrate or thiols as reducing/stabilizing agents[5-6]. In these cases, byproducts and adsorbates that may affect negatively the quality of the colloid are often present. Biosynthesized colloidal gold nanoparticles are remarkably attractive in several biomedical applications, due to their unusual physico-chemical properties, facile surface modification, and high biocompatibility[7]. However, chemical purity of these colloids is usually affected by the massive presence of biological matrices, which generally compose the synthesis medium (culture broth in case of microorganism-assisted biosynthesis, vegetal extracts, etc.). Moving to physical methods, laser ablation in liquid is a very promising technique for the synthesis of naked aqueous AuNP colloids although the reason of their stability in absence of stabilizers is yet matter of debate. Ultrapure AuNPs can be easily produced in water and many other solvents, having a fine control on morphological and physicochemical properties by changing laser experimental parameters[8,9]. Coming back to wet-chemical approaches, recently, few research groups have proposed the use of a solid reductant to assist the synthesis of AuNPs[9-12]. In this novel approach, stainless steel can be used as removable reducing substrate, in the aqueous preparation of gold nanocolloids. This innovative bottom-up method is very easy, quick and cost-effective, allowing the synthesis of highly stable NPs without any additional organic capping agent. However, the mechanisms behind this reaction are not yet fully understood. Very recently, we evaluated the effects on this system of several parameters (e.g. reaction time, precursor concentration, ionic strength, steel composition, etc.). In this keynote communication, we will provide an overview of the aforementioned approaches and we will critically evaluate their performance level in terms of AuNPs composition and properties (as per detailed spectroscopic and morphological analyses). Analytical applications of AuNPs will be thoroughly discussed, as well.

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An Integrated Modelling Approach to Study the Impacts of Nutrients on Coastal Aquatic Ecosystems in the Context of Climate Change

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Increase of atmospheric greenhouse gases (GHGs) due to anthropogenic activities is causing changes in Earth's climate. Global mean temperatures are expected to rise by 0.3 to 4.8 °C by the end of the 21st century, and the water cycle to be affected by changes in global atmospheric moisture. Coastal waterbodies such as estuaries, bays and lagoons, together with the ecological and socio-economic services they provide, could be most affected by the ongoing changes on climate being subjected to the combined changes in the physico-chemical processes of atmosphere, upstream land and coastal waters. Particularly, climate change is expected to alter phytoplankton communities by changing climate and environmental drivers such as temperature, precipitation, nutrient loadings and concentrations, and to exacerbate the symptoms of eutrophication events such as hypoxia, harmful algal blooms and loss of habitat. A better understanding of the links between environmental drivers and phytoplankton communities is necessary for predicting climate change impacts on aquatic ecosystems. In this context, the integration of climate scenarios and environmental models is a valuable tool for the investigation and prediction of phytoplankton ecosystem dynamics under climate change conditions. In the last decade, the impact of climate change on the environmental distribution and cycling of nutrients, including effects on aquatic ecosystems, prompted modeling studies at a catchment scale, mainly related to lake ecosystems. The further development of integrated modeling approaches to other types of waterbodies such as coastal waters can be a useful contribution to increase the availability of management tools for ecological conservation and adaptation policies. Here we present the case study of the Zero river basin (ZRB) in Italy, one of the main contributors of freshwater and nutrients loadings to the salt-marsh Palude di Cona (PDC), a waterbody belonging to the lagoon of Venice. To predict the impacts of climate change on nitrogen (N) and phosphorus (P) loadings of the ZRB and the consequent effects on the coastal phytoplankton community of PDC, we applied an approach integrating an ensemble of GCM-RCM climate projections, the hydrological model SWAT and the ecological model AQUATOX. Climate scenarios for the area indicated an increase of precipitations in the winter period and a decrease in the summer months, while temperatures show a significant increase over the year. The hydrological model SWAT predicted changes in water discharge and nutrient loadings of the ZRB, pointing out an increase in the winter period and a reduction during the summer months for both parameters. Simulations with AQUATOX predicted changes in water temperature, nutrient concentrations and N:P ratios, and consequent variations in the biomass and composition of phytoplankton in PDC, with major changes observed in the spring-summer period, where the abundance of warm-adapted species increases noticeably[1].

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Photoinduced Processes in Surface Waters: A Photochemical Mapping of Sweden

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Photochemical reactions induce the self-depuration of surface waters, particularly against polar biorefractory pollutants that include many emerging contaminants[1]. Photoreactions are divided into direct photolysis and indirect photochemistry. Direct photolysis involves sunlight-absorbing pollutants, and transformation is triggered by photon absorption. Indirect photochemistry takes place when sunlight is absorbed by naturally occurring compounds called photosensitisers (e.g., nitrate, nitrite and chromophoric dissolved organic matter, CDOM) to produce reactive transient species such as the hydroxyl ($^\bullet OH$) and carbonate ($CO_3^{\bullet-}$) radicals, singlet oxygen ($^1O_2$), and CDOM triplet states ($^3CDOM^*$)[2,3]. We have recently modeled photochemical processes in surface waters[4], predicting the phototransformation kinetics of pollutants and deriving photochemical parameters from available time series of water chemistry. In the latter case, one can get insight into the impact of climate change over photochemistry[5]. Here we show the application of photochemical modeling to data from a large geographic region. Based on a wide dataset that includes over 1000 Swedish lakes with their average depth, water chemistry and the incident sunlight irradiance, we could predict the steady-state [$^\bullet OH$], [$CO_3^{\bullet-}$], [$^1O_2$] and [$^3CDOM^*$]. This is the first ever photochemical mapping of a country's inland waters. The model results were validated by comparison of available field data with the predicted photochemical lifetimes of carbamazepine and ibuprofen in Lake Boren[6]. Based on the photochemical map, and compared to temperate lakes, the CDOM-rich boreal lakes of Sweden are dominated by $^3CDOM^*$ processes (except for the lakes located in the NW part of the country). In contrast, the efficient scavenging of $^\bullet OH$ and $CO_3^{\bullet-}$ limits the importance of the relevant reactions. The $^3CDOM^*$ processes are particularly effective towards the degradation of phenols, phenylurea herbicides and sulfonamide antibiotics[7]. CDOM is a key photosensitiser in most Swedish lakes and strongly prevails over nitrate and nitrite. Climate change is expected to produce water browning in boreal lakes including the Swedish ones, due to export of light-absorbing organic compounds from soil to surface waters[8]. Browning enhances the levels of CDOM, which is the $^3CDOM^*$ precursor. Therefore, the importance of the $^3CDOM^*$-induced processes is expected to increase even more in the future, in Swedish lakes as well as in many other boreal areas.
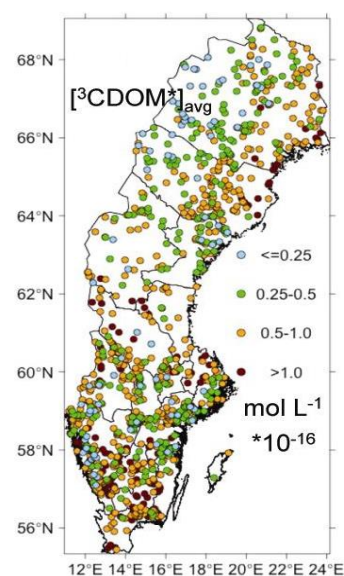
Modelled steady-state [$^3CDOM^*$] in the 1048 Swedish lakes under study

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Primary and Secondary Carbonaceous Species in PM10 and PM2.5 Samples

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Carbonaceous PM is composed of a refractory component, commonly called elemental carbon (EC), and an organic fraction, called organic carbon (OC). Organic aerosols represent a large fraction of atmospheric aerosols. The organic fraction can include saturated and unsaturated aliphatic compounds, aromatic compounds, alcohols, ketones, aldehydes, carboxylic acids, amines, sugars, polyols, and organic sulfur compounds[2]. Organic and elemental carbon were measured in daily PM10 and PM2.5 and 6 hours range time PM2.5 samples collected from September 2015 to October 2015 in a coastal rural site near Brindisi in the Apulia region (Italy), in order to determine factors affecting the carbonaceous aerosol variations. OC and EC were measured on Sunset Laboratory Thermal–Optical Carbon Aerosol analyser using NIOSH protocol.

Carbon content (total carbon TC) represented a considerable fraction for both PM10 and PM2.5. The highest values for both parameters were recorded when the winds blows from NE Europe and when Sharan Dust events have been recognized. The results show that OC and EC exhibited higher concentrations during the night hours, suggesting that stable atmosphere and lower mixing play important roles for the accumulation of air pollutants and hasten condensation or adsorption of volatile organic compounds.

Moreover, ATR-FTIR analysis were performed on some samples with the lowest and the highest OCsec and on samples characterized by Saharan Dust event. FTIR spectroscopy provides absorption peaks which can be identified and assigned to chemical bonds to identify functional groups in a complex sistem. The FTIR method is relatively simple and rapid and requires only small sample size. To our knowledge, there are only few examples of recent studies which are subjected to quantification or qualification of chemical species of aerosol samples. FTIR coupled with attenuated total reflectance (ATR) is a new technique developed recently. ATR FTIR analysis were carried out and it let identify organic functional groups included non-acid organic hydroxyl C-OH group (including sugars, anhydrosugars, and polyols, herein indicated as alcohol group), aromatic C=C-H group, aliphatic unsaturated C=C-H group, aliphatic saturated C-C-H group, non-acid carbonyl C=O group, carboxylic acid COOH group, and amine NH2 group. Among the inorganic ions, carbonates, sulfate, silicate and ammonium can be recognized.

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Cutting-edge developments in shotgun proteomics, peptidomics and shotgun phosphoproteomics in real matrices

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The keynote will give an overview of some applications of shotgun proteomics, and related technologies, in cutting-edge research fields. In particular, the potential of shotgun proteomics in shedding light on the interaction between nanomedicine vehicles and biological systems will be discussed. By studying the nature and composition of the proteins adsorbed onto such nanoparticles it becomes possible to understand the fate of the nanoparticles themselves and of their loaded drug, if the vehicles will reach the target cells or be cleared due to opsonization. Key case studies will be discussed, in particular to better understand the effect of a popular anti-opsonization strategy, i.e. PEGylation, and the use of model animal systems, such as mice, during nanomedicine vehicle development stages.

A brief overview of peptidomics will be provided. Initially considered a subfield of proteomics, peptidomics is demonstrating to be much more important than that, as an individual discipline to which ordinary proteomics technologies need to tailored for improved identification and to tackle common drawbacks due to endogenous peptide extraction, separation and unrestricted database search space; in particular, its potential in discovery of bioactive peptides from food and waste matrices will be considered.

Finally, the use of peptidomics in post-translational modification analysis will be discussed. General considerations on method development for phosphopeptide analysis will be presented, with a special attention to the investigation of complex real matrices and endogenous phosphopeptides characterization in samples of biological significance.

A New Magnetic Graphitized Carbon Black TiO2 Bomposite for Phosphopeptide Selective Enrichment in Shotgun Phosphoproteomics

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Protein phosphorylation is a dynamic and reversible post-translational modification that is widely used in biological sys-tems to regulate protein-protein interactions and thus, to rap-idly activate or deactivate signaling pathways. It has proven to play a key role in several biological processes, such as cellu-lar signaling and communication,1 cell adhesion and migra-tion, transcriptional and translational regulation, cell division and apoptosis.4 Gaining knowledge about these pathways is strongly related to cell activity and it may help shedding light on relevant physiological and pathological conditions. In this sense, proteomics technologies and mass spectrometry (MS) have become the leading tools for the study of transient post-translational modifications. However, despite the great poten-tial of HPLC-MS/MS in the field, direct analysis is generally not possible without prior enrichment of target proteins or peptides[1]. In this context, the development of new materials for use in phosphoproteomics is of great demand to fill the existing gaps and to allow for a comprehensive analysis. Popular methods for phosphopeptide enrichment consist of stationary phases in batch format, and self-made or commercial micro-columns packed into pipette tips. Some advantages over miniaturized column format can be found by using dispersive solid-phase magnetic separation. With respect to packed micro-columns, the use of magnetic phases avoids the step of column packing[2]. Graphitized carbon black (GCB) has been employed for extraction of several classes of analytes, due to the large surface area and the unique chemistry of its surface groups that allows to extract a wide range of analytes, including polar, acidic compounds. Despite the fact that structurally-related materials, such as graphene, found application as hybrid-components in phosphoproteomics, surprisingly, GCB has never been used for the selective enrichment of phosphopeptides. For this purpose, in the present work we used GCB to prepare a magnetic composite with TiO2 (mGCB@TiO2) that was then applied to yeast total extracts. We exploited the high surface area provided by nanostructures, the presence of nano-TiO2 for selective binding of phosphopeptides, and the magnetic responsiveness of magnetite for solid-phase separation. The material was extensively characterized at each modification step by transmission electron microscopy, Fourier-transformed infrared spectroscopy, thermogravimetric analysis, Raman spectros-copy and porosimetry. Next, the new system was applied for the enrichment of casein phosphopeptides from a simulated tryptic digest with bovine serum albumin (BSA:casein, 100:1). Finally, after assessing the potential applicability, the composite was em-ployed for enriching phosphopeptides from yeast protein digests. This allowed us not only to optimize the enrichment protocol, but also to fully compare its performance to commercial TiO2 spin columns. To achieve this aim, the optimized enrichment protocol was included in a typical shotgun proteomics analytical workflow based on nanoHPLC-MS/MS analysis.

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pH-Controlled Assembly of DNA Tiles

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We demonstrate a strategy to trigger and finely control the assembly of supramolecular DNA nanostructures with pH. Control is achieved via a rationally designed strand displacement circuit that responds to pH, and activates a downstream DNA tile self-assembly process. We observe that the DNA structures form under neutral/basic conditions, while the self-assembly process is suppressed under acidic conditions. The strategy presented here demonstrates a modular approach towards building systems capable of processing biochemical inputs and finely controlling the assembly of DNA-based nanostructures under isothermal conditions. In particular, the presented architecture is relevant for the development of complex DNA devices able to sense and respond to molecular markers associated with abnormal metabolism[1].

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Comunicazioni Orali

A Validated SIM GC/MS Method for the Simultaneous Determination of Linear Polyalkylsiloxanes OH-*terminated* in Octamethylcyclotetrasiloxane

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Optical fibers presently on the market are mainly manufactured by drawing from a cylindrical preform of silica[1]. Octamethylcyclotetrasiloxane ($[-Si(CH_3)_2O-]_4$) is the most commonly used liquid feedstock for high purity SiO_2 production performed at 180°C, according to the following reaction:

$$[-Si(CH_3)_2O-]_4 \ + \ 16 \ O_2 \ \xrightarrow[180°C]{\Delta} \ 4 \ SiO_2 \ + 8 \ CO_2 \ + 12 \ H_2O$$
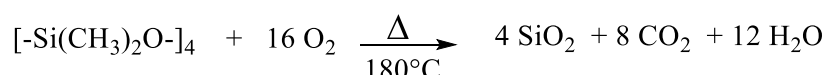
Octamethylcyclotetrasiloxane feedstocks usually contain impurities deriving from the production process[2,3,4], as cyclic molecules possessing 3 to 8 silicon atoms and linear polyalkylsiloxanes OH-*terminated* (L_n, with $3 < n < 12$), having the formula:
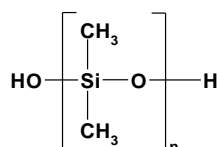
In particular, linear polyalkylsiloxanes with $n > 6$, due to their high boiling point, form polymeric compounds[5], with consequent deleterious deposits in the vaporization and conversion equipment of the chemical plants[3].

In order to avoid serious problems to the silica glass manufacturing process and to maintain the fiber quality, the use of high purity siloxane feedstocks (total impurities < 0.25 *ppm*) is mandatory[3]. For this purpose, there are various processes that involve their purification[3,6]. To that end, the analytical determination of the high boiling impurities constitutes a crucial task.

In this communication will be discussed a new and high sensitive method to determine all the polyalkylsiloxanes OH-*terminated* contained in octamethylcyclotetrasiloxane feedstocks. Preconcentration and pretreatment, followed by mass spectrometric analysis in SIM mode, allowed the determination of these impurities with a detection limit of 50 *ppb*. The proposed method was applied to several octamethylcyclotetrasiloxane samples.

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Identification of Urinary Biomarkers of Meat and Dairy Products Intake by Means of UHPLC-QTOF/MS Untargeted Metabolic Profiling

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Meat intake has been associated with positive as well as negative health outcomes. The nutritional importance of meat is related to the content of high quality proteins and other micronutrients essential for growth and development. On the other hand, red meat consumption has been associated to increased risk of colorectal cancer and, possibly, cardiovascular diseases, mortality and type 2 diabetes. However, a causal relation between red meat consumption and negative health outcomes is still controversial[1]. In order to shed light on this issue a reliable assessment of red meat intake is needed. Meat intake has often been assessed using 24-h recalls, weighed food diaries, and food frequency questionnaires[2], although they have been shown to be very imprecise methods[3]. Dietary biomarkers measured in biological fluids have been proposed as possible objective measurements of the actual intake of specific foods. Metabolomics offers a potential tool in the area of nutritional assessment[4] allowing measurement of a range of small molecules and identification of potential dietary biomarkers present in urine and blood. A meat intake marker should reflect dietary meat content and, at the same time, should be able to differentiate meat consumption from that of other protein-rich food such as dairy products. In this study, the identification of urinary meat and dairy exposure markers was accomplished after ingestion of red meat and dairy meals containing equal amounts of macronutrients. A randomized, controlled, cross-over single meal study was conducted in 17 subjects. In total, 48 hours urine samples were collected. This included urine samples collected 24 hours prior to ingestion of the test meal and urine samples collected during the first 2 hours, from 2 to 4 hours and from 4 to 24 hours after ingestion of the test meal. An ultra-high-performance-liquid chromatography coupled via electrospray (ESI) source to a Q-TOF mass spectrometer was used for separation and the metabolic profiling of the samples. To evaluate the effect of the meal over time on urine metabolome a linear mixed model was fitted for each detected feature. Twenty-four hours urine samples were analysed by multivariate data analysis by means of two different approaches: at first data were analysed by Partial Least Squares Regression (PLSDA), and subsequently data were investigated by multilevel-PLSDA to correct for the random effect of the subjects. The 78 compounds resulting significant for discriminating the two diets in both analysis have been selected as meat or dairy products intake biomarkers. The selected marker was fragmented to accomplish their chemical identification. Several markers for meat and dairy products intake were tentatively identified based on the accurate mass, fragmentation spectra and by means of comparison with available databases. Some of them were synthesized to allow the identity confirmation. Combinations of these markers are proposed as specific markers for meat or dairy product intake. However, further identity confirmation with authentic standard compounds and further validation in larger study settings are needed for several of the markers.

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On-line SPE-LC-MS/MS Analysis of Wide-Range Polarity Pharmaceutical Compounds in Aqueous Matrices: Problems and Solutions

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Modern environmental analytical chemistry must deal with the increasing demand of high-throughput analysis. Furthermore, the environmental monitoring of organic micropollutants in water samples is a fairly laborious issue, due to a number of reasons, such as the need of the simultaneous determination of a very high number of compounds, frequently characterized by a wide range of physicochemical properties and capable to exert negative effects on the environment, even at very low concentrations (i.e. parts per billion-trillion levels). All these issues can be faced using on-line solid phase extraction coupled to tandem mass spectrometric detection (on-line SPE-LC-MS/MS), which has been successfully applied to the monitoring of several classes of organic micropollutants in various aqueous matrices[1,2]. Pharmaceutical compounds in aqueous matrices are matter of environmental concern owing to their widespread use, continuous release, persistence, and increasing evidence for their ecotoxicological effects[3]. Pharmaceuticals are excreted either as such or as metabolites after human administration and via the sewage system they reach wastewater treatment plants (WWTPs)[4] where they are often inefficiently removed, thus being discharged into the receiving water bodies[5]. Pharmaceuticals represent a group of substances characterized by the presence of acid and/or basic functionalities and a very wide range of polarity, thus representing a challenge for the analytical chemist when a multiresidual determination is required. In this work, the applicability of the on-line SPE-LC-MS/MS approach to the determination of a number of pharmaceutical compounds characterized by very different physicochemical properties was investigated. Antibiotics, beta-blockers and anti-inflammatory drugs most used in Italy were selected according to the reports of the Italian Medicine Agency, together with some metabolites. Different SPE sorbents, such as the poly(divinylbenzene-co-N-vinylpyrrolidone) (PVB-VP) and PVB-VP functionalized with a weak cation exchange group, and stationary phases (e.g. octadecylsilane and pentafluorophenyl silane) were tested. Under the best experimental conditions, recoveries included in between 40 and 120% were obtained on MilliQ water. Apparent recoveries significantly differed from these values, mainly due to matrix effects within the ionization process. Detection limits were however included from sub-ng/L to tens of ng/L, depending on the compound and sample analysed. The overall analysis time per sample was about 25 minutes. The analysis of various effluent WWTP samples evidenced the presence of several pharmaceuticals and especially antibiotics and beta-blockers, at concentrations as high as tens of µg per litre.

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Insights into Structure-Bioaccessibility Relationships of Polyphenols in Red Chicory by HPLC-MS/MS

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Two varieties of red chicory from southern Po Delta area have been characterized in terms of polyphenols content. Attention has been mostly paid to phenolic acids and flavonoids[1]. Qualitative and quantitative determinations have been performed on sample extracts by HPLC-MS/MS. Both glycosylated and ester forms of phenolic acids as well as glycosylated flavonoids were mainly identified. Seven target bioactive compounds were selected and their bioaccessibility from red chicory vegetable matrix was investigated through simulated in-vitro static digestion process[2,3]. This gastro-intestinal model uses simulated fluids (i.e., salivary, gastric and duodenal) based on electrolytic solutions of different physiological enzymes miming the stages of digestion process. Fractions of polyphenols released from the vegetable matrix were quantitatively determined. Bioaccessibility results show well defined behaviors associated with both specific structures of aglycone or phenolic acid and position of O-glycosyl moiety. Dicaffeoyltartaric acid together with apigenin-7-O-glucoside and kaempferol-7-O-glucoside have similar bioaccessibility trend that is characterized by a higher value for duodenal phase than for gastric one. Quercetin-3-O-(6"-O-malonyl)-glucoside behave in reverse way and gastric bioaccessibility turns higher than duodenal. Lastly, the difference between gastric and duodenal bioaccessibility is ehnanced in the case of cyanidin-3-O-glucoside and two isomers of caffeoylquinic acid. Advanced investigations involved also living biological systems (i.e., cellular lines) and they were aimed at understanding effects of polyphenols on degenerative diseases. Particularly, investigation of the role of polyphenol metabolites and their positive synergic effects together with other nutraceutics was promising for uncovering hidden aspects of bioactives mode of action and their health effects.

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Shotgun Proteomics for Detecting Seafood Fraud

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Nowadays, there is an increasing interest on food safety and, in consequence, fighting food fraud has become very important. The development of new analytical technologies, as well as the application of the existing ones, can be very helpful to detect fraud in the seafood commerce. In particular, the discrimination between fresh and frozen-thawed seafood is very important to protect consumers. The selling of frozen-thawed fish labeled as fresh it also represents a potential risk for the consumer's health related to microbiological safety: when freezing-thawing cycle are repeated, it leads the activation of residing dormant micro flora increasing the total microbial count.

In this research, we employed a shotgun proteomic approach for the detection of seafood fraud. A new experimental protocol was developed for the extraction of proteins from tissue samples of curled octopus (Eledone cirrhosa). Through the use of the high-resolution LC-MS/MS for the reliable and fast quantitation of proteins, we were able to identify new biomarkers for the differentiation between fresh and frozen-thawed octopus.

In conclusion, we demonstrated that shotgun proteomics could have a great impact on the rapid screening of seafood safety.

In Vitro Meningeal Permeation of MnFe$_2$O$_4$ Nanoparticles

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MnFe$_2$O$_4$ nanoparticles (NPs) are commonly produced in some occupational settings and may reach high concentration in activities such as arc-welding or ferroalloy metallurgy. Manganese is an essential cofactor in enzyme activities but it has been demonstrated that long-term exposure to excessive levels can lead to "manganism", a neurodegenerative disease resembling Parkinson features[1]. The same symptoms have been described in rats exposed to MnO$_2$ NPs[2]. Inhaled NPs deposit partially in pharynx and nasopharynx and may reach the central nervous system through the olfactory nerve, which is completely enveloped by the meningeal membranes throughout its course from the nasal cavity to the olfactory bulb. The aim of the present study was to assess the permeability properties of meningeal membranes, currently undefined, towards MnFe$_2$O$_4$ NPs, which represent a common by products in the arch-welding fumes[3] and may exert chronic neurotoxic effects on humans.

This study investigated in vitro the transmeningeal absorption of 50 nm MnFe$_2$O$_4$ NPs, using excised porcine meninges mounted on Franz diffusion cells. Two donor solutions have been tested: the first containing MnFe$_2$O$_4$ NPs (2.0 g/L) and the second obtained by the ultrafiltration of the first one with centrifugal ultrafilter (MWCO 10 kDa), in order to test only the NPs water soluble fraction[4]. At selected intervals 1 mL of the receiving bathing solution was withdrawn and collected for the analysis, and immediately replaced with an equal volume of fresh made physiological solution. At the end of the experiment the meninges pieces were removed, washed and mineralized for the following analysis. Each experiment was carried separately for 4 hours. Inductive Coupled Plasma Mass Spectrometry was used to determine the total manganese and iron concentrations in the receivingphases (ion mass selected: 55 u.m.a. for Mn and 54 and 57 u.m.a. for Fe). ^{103}Rh has been used as internal standard for all the samples. The analysis was carried out in kinetic energy discrimination (KED) mode. The total iron and manganese concentrations in the donor phases and in the solutions resulting from the tissue mineralization were performed by Inductive Coupled Plasma – Optical Emission Spectrometry. The measurements were conducted at the operative wavelengths of 238.204 nm for Fe and 257.610 nm for Mn. Results (expressed as mean and SD) showed that Mn flux permeation through the meninges could not be calculated, since only trace of the metal was found in receiving solutions of cells exposed to MnFe$_2$O$_4$ NPs (5.5 ± 4.4 ng/cm^2), ultrafiltered solution (3.5 ± 3.2 ng/cm^2) and blank cells (2.1 ± 1.2 ng/cm^2). Differences did not reach the statistical significance.

This study shows that MnFe$_2$O$_4$ NPs penetrate the meningeal membrane in a non-significant amount, thus making unlikely the hypothesis of a transcellular and paracellular absorption through the olfactory nerve but but not excluding the hypothesis of an intraneuronal absorption.

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Identification of MT-45 Metabolites: *in silico* Prediction, *in vitro* Incubation with Rat Hepatocytes and *in vivo* Confirmation

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The new psychoactive substances (NPS) do not represent a novelty in the drug market anymore, in fact the phenomenon has been largely reported and monitored worldwide since the late nineties[1]. The use of these substances is associated with dissociate mental states and psychedelic sensations; however because of the lack of legal restrictions to their marketing, these drugs are easily available. These pharmacological effects and the difficulty in detecting the parent compound in urine, highlight the importance of metabolite identification for developing analytical methods for clinical and forensic investigations. The aim of this work was to ascertain the chemical structures of the metabolites of MT-45, in order to allow further pharmacological studies and to better assess the health implications of the abuse of this substance[2]. MT-45 is a synthetic opioid firstly developed in the early 1970s by the Japanese company Dainippon Pharmaceutical Co. Ltd, it has a pharmacological activity comparable to morphine and it has been involved in intoxications and fatalities reported in Europe and in USA[3]. It was recently subjected to control measures, but until now, the metabolic pathways of the substance were still unknown[4]. When metabolism is unknown, determining targets for analysis is critical, but using rat hepatocytes and LC–HRMS, 14 novel phases I and II MT-45 metabolites were identified: products of monohydroxylation, dihydroxylation, and N-dealkylation; glucuronide conjugation of mono- and dihydroxylated metabolites as suggest before by Scheidweiler et al for several synthetic cannabinoids[5]. The detected metabolites were firstly predicted *in silico*, then incubation of the drug with rat hepatocytes was carried out and the obtained metabolites were identified by LC–HRMS, with retention times, mass shift between theoretical mass and observed mass (<10 ppm), peak abundance and fragmentation pattern. Hydroxylated MT-45 was found to be the major metabolite of MT-45 *in vitro* experiments. The presence of all metabolites was confirmed by *in vivo* experiments in urine samples of CD-1 male mice; in these samples hydroxy-MT-45 glucuronide and di-hydroxy-MT-45 glucuronide are the most abundant metabolites, while the parent drug is found at concentration < 10 ng mL^{-1} after 300 min. The knowledge of phase I and II MT-45 metabolite structure is then crucial to develop analytical methods to identify MT-45 consumption in clinical and forensic testing.

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All PEDOT:PSS Electrochemical Transistors as a Platform for Sensing

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Organic electrochemical transistors (OECTs) are devices which find growing interest in the field of biological and chemical sensing. Although the OECT transduction is based on electrochemical reactions, the transistor architecture offers several advantages respect to amperometric sensors such as signal amplification, the use of an easy and cheap readout electronics, low supply voltage (usually < 1 V), low power operation (< 100 µW), bio-compatibly and, moreover, they can be easily miniaturized and adapted to non-flat, flexible and even textile devices[1]. This contribution want to report the potentiality of such devices by describing an OECT based only on PEDOT:PSS as conductive material, that can be exploited to obtain low cost sensors[2,3] with very high performance. The sensor was optimized by studying the response to different redox compounds of biological interest such as ascorbic acid, dopamine, adrenaline and uric acid. The bio-molecules react with PEDOT:PSS by extracting charge carriers from the transistor channel, and consequently an increase of analyte concentration leads to a decrease of the absolute value of the drain current.
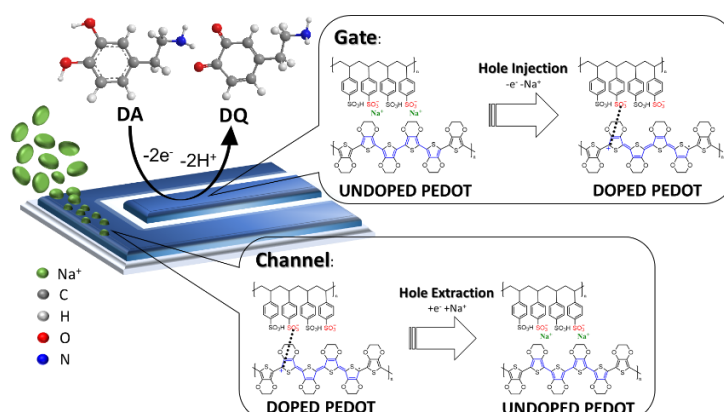
The main drawback of such devices is the lack of selectivity which hinders its wide use in real applications. To address this issue, we use a dual approach: on one side we have implemented a potentiodynamic approach that exploits the variation of the operating gate bias voltage in order to obtain a trans-conductance curve wherein the waves due to different redox compounds are separated. On the other side, the physical and chemical modification of the PEDOT lactice of the gate electrode offers great opportunities for the implementation of a selective sensor.

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Development of enzyme-based microsensors for *ex vivo* analyses

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Nowadays real-time quantitative measurements of many proteins, metabolites and cancer biomarkers, are feasible by the use of biosensors for *in vitro* and *in/ex vivo* studies[1]. Although, strategies on biosensor design are rapidly increasing, enzymatic biosensors remain a large and important field of study. Enzymes achieve molecular recognition of the substrate based on structural complementarity and exploit an analyte-dependent step to convert an electrochemically inert substrate to an active product, often revealing high selectivity, sensitivity, time scale and information content. Even if, amperometric biosensors have been around since the early 1960s[2,3], it is the production of very tiny micro-electrode biosensors that offers great utility for studying chemical signalling for *in vitro* (at single cell level) and *in/ex vivo* studies. These microelectrode biosensors have the advantage of offering a better spatial and temporal resolution and they are considerably less invasive than other electrode (i.e. microdialysis electrodes)[4]. The miniaturization of biosensors is challenging, as they need to be both extremely small and highly sensitive. In fact, smaller is the sensor, smaller is the electrode surface area, and consequently the recorded amperometric signal diminishes[1]. The requirement in miniaturizing biosensors is to maintain a signal to noise ratio that enables effective and sensitive detection of the analyte. The tiny sensing surface of the sensor has to be coated with a high density of enzymes in their native active conformation. Furthermore, for *ex vivo* analyses, it is mandatory to control the selectivity of the signal and the mechanical strength of the sensor itself, which has to enter in tissues without damaging.

In this work, we present the development of small, robust and highly active micro-electrode enzymatic biosensors for detecting metabolites as glucose, lactate and ATP from single living cells to tissues analysis., such

7μm x 200μm
carbon fiber
enzyme-based
micro-biosensor
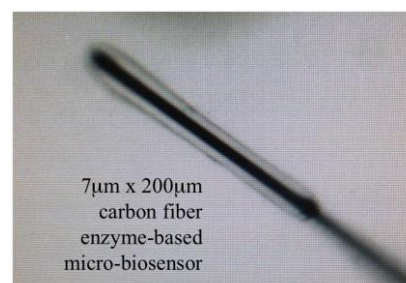
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Potential and Limitations of Voltammetric Measurements for the Characterization of Electrode Surface

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In this work, we tested the possibility to use voltammetry for the evaluation of the area of solid electrodes. In the first step of the research study we adopted two voltammetric techniques, well known in the literature: i) cyclic voltammetry (CV) at different scan rates, followed by processing of the peak height with the Randles-Sevcik equation; ii) linear sweep voltammetry (LSV) at different electrode rotation rates, followed by data treatment with Levich equation[1,2]. The ferrocene/ferrocenium redox system was chosen as a probe for its well-known reversible process of diffusion-controlled single electron transfer. Acetonitrile and tetrabutylammonium esafluorophosphate were used as solvent and supporting electrolyte respectively. Initially attention was focused on a solid gold electrode (SGE). The evaluation of its area was made both on the electrode as received, without applying any treatment, and after a drastic surface treatment, to compare the effect of mechanical polishing on the measured area. The results obtained showed that such a treatment gave rise to a significant decrease of the standard deviation of the results and a decrease of the value measured for the area. Method i) was then applied to a glassy carbon electrode (GCE) in three different conditions: unmodified and modified with an Au-film (AuF-GCE) and with an Au-nanoparticle layer (AuNP-GCE), to investigate the influence of the "roughness" of the electrode surface on the value measured for the area. According to the literature, the above-mentioned electrochemical techniques should respond to the electrode projected areas[2,3]: however, we found that the measured areas differ for each modification, suggesting that the morphology of the active surface influences the results of the calculation. Subsequently, method i) was applied to home-made carbon paste electrodes (CPE) of different sizes, to test the relationship between the diameter of the electrode body and the measured area. The calculated areas were compared with those obtained by recording electrode images by scanning electron microscopy (SEM) and processing the data with Wolfram Mathematica software. In the second step of the study, the actual active surfaces of the SGE and of the two Au-modified GCEs were estimated by CV in H_2SO_4, from the areas of the cathodic peak obtained in each voltammogram, through the theoretical charge associated with the reduction of the oxide layer per unit surface area of gold[4]. In conclusion, voltammetry provides information on the area of solid electrodes, but, despite the notoriety of the equations considered in this study, there are some doubts about the kind of "area" actually involved in the measurement process; further studies are required to correctly interpret experimental results. Finally, the evolution of the SGE surface during an extended period of usage was checked by periodically recording cyclic voltammograms in H_2SO_4. Clear differences were observed in the CV curves over time and a relationship between the shapes of the voltammograms and the performance of the electrode was found: therefore, CV can be a used to monitor the state of the SGE surface and identify the need for a mechanical polishing or even a substitution of the electrode.

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Development of an Electrochemical Magneto-Immunosensor for the Detection of *Campylobacter*: A Preliminary Study

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Campylobacter is an important pathogen genus that cause acute bacterial diseases in humans worldwide. Most infections (about 90%) are caused by *C. jejuni* species, less frequent are those caused by *C. coli*, while sporadic cases are caused by *C. lari* species and *C. upsaliensis*. Outbreaks of Campylobacteriosis have traditionally been linked to the consumption of food products of animal origin (e.g. meat and eggs) but, more recently, an increasing number of outbreaks have been associated to contaminated fruits and vegetables, especially raw leafy green vegetables[1]. Conventional bacterial testing methods rely on specific microbiological media to isolate and enumerate viable bacterial cells in food. These traditional methods are very sensitive and inexpensive, but require several days to generate results because they rely on the ability of microorganisms to multiply to produce visible colonies. Therefore, rapid, sensitive and specific methods to detect pathogens are needed. In this work a sandwich ELIME (Enzyme-Linked Immuno Magnetic Electrochemical) assay to detect *Campylobacter* is reported. The proposed system is similar to that already developed by our research group for *Salmonella enterica*[2,3]. Magnetic beads (MBs), coupled to a strip of eight-magnetized screen-printed electrodes, effectively supported a sandwich immunological chain. Enzymatic by-product is quickly measured by differential pulse voltammetry, using a portable instrument. Different antibodies, blocking agents and treatments for bacterial cultures were tested. The final system employs: MBs tosyl-activated blocked with dry milk and coated with a capture polyclonal antibody anti-*Campylobacter*, whole *Campylobacter* cells (inactivated with NaN_3) and a detection antibody conjugates with ALP enzyme. For a simple and rapid assay, the coating and blocking steps were performed in a preliminary phase, while the two sequential incubations for the immuno-recognition events were merged in a single step of 1 h. The final proposed conditions are those that ensured the best analytical performance for *C. coli*, while, to date, there are still difficulties to detect *C. jejuni*. The future work will focus on improving the response towards *C. jejuni* and the next application of the assay in contaminated matrices, such as irrigation water and vegetables, object of various community alerts in the last few years.

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Silica-Based Composites for the Consolidation of Earthen Materials

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Earthen materials are largely used in the architecture of many areas of the world, but the lack of well-established processing techniques and recipes make their conservation challenging. The object of this research is to develop a compatible and long-lasting consolidating treatment for adobe bricks, unbaked mixtures of soil and water, often added with organic matter and/or lime. We are experimenting the formulation and application of a silica-based system, where the combination with a cellulosic derivative and nano-lime aims at obtaining greater effectiveness and compatibility with the substrate.

Mesoporous silica nanoparticles were prepared following a synthesis[1] selected to obtain adequate particle size and porosity (250 nm uniform spheres with 300-600 m^2/g BET surface area were observed with SEM, DLS and porosimetry). Various amounts of *Klucel®-G* (hydroxypropyl cellulose ethanol solution) and *NanorestorePlus®* ($Ca(OH)_2$ nanoparticles ethanol dispersion) were added to the silica aqueous dispersion and kept under stirring to maximize the interaction. Components proportions were chosen according to common restoration applications and in order to minimize the aqueous content. The systems obtained were characterized by DLS, Z Potential, turbidimetry, TGA, FT-IR and SEM-EDS.

Turbidimetric tests confirmed the expected stabilizing effect of hydroxypropyl cellulose on silica, while almost instantaneous flocculation occurs in $Ca(OH)_2$-silica systems, in accordance with surface charge measures. Interestingly, the ternary system maintains a significant stability over several days. FT-IR analyses pointed out an intensification of the stretching band at 965 cm^{-1}, characteristic of calcium silicate hydrate[2,3], in all the systems containing silica and $Ca(OH)_2$ nanoparticles. The hypothesis of the formation of a cementing phase is supported by SEM observations of similar structures. Further analyses are underway. Following these first results, one of the ternary formulations was selected for preliminary application tests on adobe samples from Mexico.

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Chemical Poly Ethyl Methacrylate (PEMA) Organogels for the Removal of Pressure Sensitive Tapes (PSTs) from Paper Artworks

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Chemical poly ethyl methacrylate (PEMA) organogels loaded with Diethyl Carbonate (DEC) were synthesized by means of radical polymerization[1,2]. These systems are specifically designed for the removal of degraded Pressure Sensitive Tapes (PSTs) from paper artworks, a very common issue in the field of manuscripts and drawings restoration. DEC, an organic green solvent, shows interesting swelling properties towards PSTs components; besides it is inert to most common inks and dyes, unlike other commonly used cleaning systems such as polar solvents and microemulsions[3].

Several organogels were obtained by changing reaction parameters; on the basis of macroscopic evaluations (e.g. mechanical properties, homogeneity), some systems were chosen for a deep physico-chemical characterization performed by means of different techniques. Thermogravimetry (DTG), Differential Scanning Calorimetry (DSC), and gravimetric analysis were used to assess solvent content and uptake/release behaviour of gels; rheological analysis permitted the evaluation of their viscoelastic properties; Fourier Transform Infrared Spectroscopy (FT-IR) and Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) allowed to investigate the presence of unwanted residues in the exchange solvents and the effectiveness in the removal of PSTs. The penetration of DEC through the backing layer of PSTs and the swelling of the adhesive was investigated by means of Laser Scanning Confocal Microscopy (LSCM).

Cleaning tests on mock-up samples and real artworks were also successfully performed.
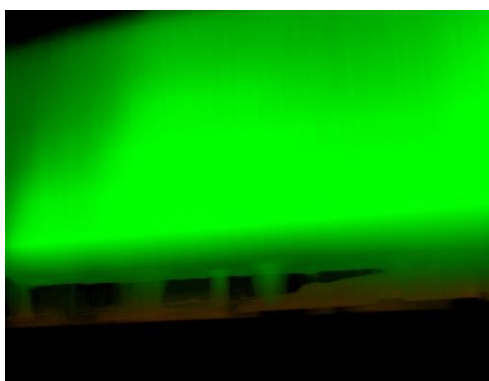
Fig. 1 – Laser Confocal image of a PEMA-DEC gel onto a PST sample: penetration of DEC (in green) through the backing up to the adhesive layer (in red)
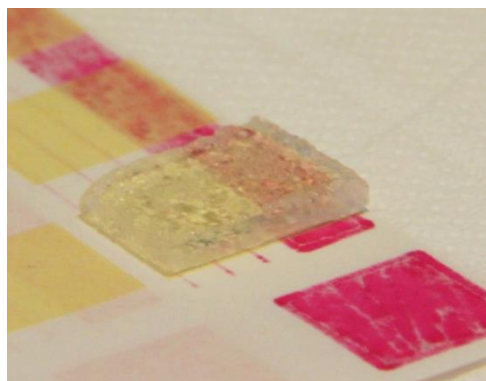
Fig. 2 – Application of a PEMA-DEC organogel for the removal of a Masking Tape from a mock-up sample

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Surface Cleaning of Artworks: Structure and Dynamics of Nanostructured Fluids Confined in a Polymeric Network

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Nanosystems and confinement tools for the controlled release of a cleaning agent, e.g., hydrogels and microemulsions, have been used for several years for the treatment of delicate surfaces in art restoration interventions. However, notwithstanding the unprecedented achievements from an applicative point of view, a fundamental comprehension of their interaction mechanism is still lacking. In this study PVA hydrogels, obtained via freeze-thaw processes, are prepared as scaffolds for water-based nanostructured fluids for application in the cleaning of artworks: rheological, thermal, microscopic and scattering techniques showed that, depending on the number of freeze-thaw cycles, the hydrogels exhibit different physicochemical and viscoelastic properties, making them suitable for application in a broad range of cleaning issues. The gels have been loaded with an oil-in-water microemulsion and the diffusion of the microemulsion droplets inside the polymeric network has been investigated through Fluorescence Correlation Spectroscopy (FCS), demonstrating that the microemulsion is permanently kept inside the matrix and can freely diffuse in the network. In addition, we show that, when the gel-microemulsion system is put in contact with a layer of hydrophobic grime, a dynamic interaction between the microemulsion droplets and the underlying layer is established, leading to the solubilization of the hydrophobic molecules inside the droplets in the gel matrix. Thus, for the first time, through FCS, insights about the removal mechanism of hydrophobic grime upon interaction with cleaning agent embedded in the polymeric matrix, are obtained. Some examples of significant case studies treated with these innovative materials will be presented.

Nanocomposites for the Consolidation and Deacidification of Cellulose-Based Artifacts

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A restoration method designed for the conservation of degraded fibrous-based artifacts should addressed the two main problems concerning these materials. Firstly, a mechanical reinforcement of artifacts is usually needed to ensure that the original material keeps its integrity. Moreover, pH buffering is required to prevent acidic degradation that is inherent to natural materials[1].

It has been recently shown that these two issues are related. For instance, it takes less than 100 years for acidic compounds to induce a significant loss of mechanical properties in canvases based on natural materials. (2) The same degradation effect of acidic compounds is found in paper-based artworks, drawings and documents. In this regard, several deacidification methods have been proposed and proven efficient in hampering the degradation of cellulose[3,4,5].

Research efforts have been recently devoted to the development of a nanocomposite material for the consolidation and concomitant pH adjustment of cellulosic works of art and artifacts that is one of the goal within the EU Project NANORESTART.

The use of nanocellulose, in combination with nanoparticles and cellulose derivatives, could ensure the consolidation of fiber-based materials using almost entirely natural materials. The choice of these materials is due to the high compatibility of the proposed treatments with the original fibrous support, which is essential from the conservation point of view.

Considering the high variability of cellulose-based artifacts, several different approaches were followed in order to provide conservators with a wide palette of tools that can be used on different works of art that are in need of both consolidation and deacidification treatments.

Nanocomposite materials were analyzed in order to gain more information about their structure and characteristics. Mechanical tests on not aged and aged reference samples were used to assess the consolidation efficacy of the proposed consolidation treatments before testing on real case studies.

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FIB-FESEM-EDX Study of Silver Roman Coins: Characterization Of The Core Microstructure And Corrosion Products With A Multi-Analytical Approach

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Metal artifacts are among the most common materials in the Cultural Heritage field: alloys have been used in several fields of everyday life and their analysis can prove useful information about the technology of the ancient populations. In particular, coins are important for studying provenance, dating, minting and corrosion processes as well as provenance and stratigraphy of the archaeological sites[1,2].

In this work a set of Roman silver coins, dated back to the Antonini's period, has been analyzed using FIB-FESEM-EDX aiming to establish the used technique for the silvering and discriminating different mints. Recently, this approach was used as a complementary technique for the characterization of bronze coins[3] but this is the first application on Roman silver coins. The aim of this work is to investigate the microstructure and the composition of the alloy with a micro-invasive approach. Commonly to investigate the composition of the metal core are used invasive and destructive techniques, therefore the developing of methods with minimal damage on metal is of a great interest.

The study of Antonini's coins is also important as in that historical period Roman Empire underwent to a severe debasement which influenced coinage, so the surface silvering it is still an open question. Diocletian in the 294 A.D. introduced a complex alloy (Cu-Sn-Pb-Ag) with an Ag-rich surface patina of 2 μm. Some coins of the set show a very fine silvered surface with a core composition entirely made of Cu, whereas others have the composition made of Sn-Cu-Pb with a very low concentration of Ag. This technique has been joined with surface analysis (Raman spectroscopy, SEM mapping, voltammetry of microparticles (VMP) and electrochemical impedance spectroscopy (EIS)) for a deeper knowledge of the samples. The results showed the leaching of the Sn and Pb as well as of the Cu that formed several common products on the surface of the coins, *i.e,* Cu_2O, CuO, PbO, SnO. The presence of $AgCl$ has been detected in several coins.

In conclusion, FIB-FESEM-EDX analysis provides information about the composition, structure and thickness of the metal patina as well as the composition and microstructure of the metallic core which in turn reflects the technology of minting. As a result, FIB-FESEM-EDX features yield information for the diagnostic, authentication, technologies and historical context in which the coins were fabricated. Such multi-analytical data allow us the possibility of screening the coins minting, obtaining significant differences between those minted in Roma and those minted in the Gallia.

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I Materiali E La Tecnica Pittorica Di Konrad Witz Nel Suo Capolavoro Di Ginevra

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Konrad Witz, tedesco di origine, ma attivo soprattutto in Svizzera fra il 1431 e il 1445 circa, è, per dirla con le parole dello storico dell'arte Jonathan Jones, "un gigante della pittura medievale che aspetta solo di essere scoperto". Le opere superstiti di questo artista sono davvero poche; fra queste sicuramente le più importanti sono le due tavole dipinte nel 1444 su entrambi i lati per l'altare maggiore della Cattedrale di Ginevra ed ora conservate nel Musée d'art et d'histoire de Genève[1]. Il presente studio illustra i risultati della campagna diagnostica condotta, prima dell'intervento di restauro, sui materiali costitutivi, la tecnica esecutiva e lo stato di conservazione di queste opere. Le indagini scientifiche sono state condotte dapprima mediante tecniche strumentali non invasive, successivamente su microcampioni prelevati in modo mirato dopo l'acquisizione dei dati delle analisi preliminari.

Gli obiettivi delle indagini sono così sintetizzati:

- osservazione in situ della superficie delle opere mediante l'impiego di microscopia a bassi ingrandimenti, in luce visibile e UV, per osservare lo stato di conservazione della pellicola pittorica originale e differenziare le parti originali dalle eventuali ridipinture;
- analisi XRF in più punti delle superfici policrome per avere dati preliminari sulla composizione degli elementi chimici e orientare la scelta dei prelievi di campione;
- esame chimico-stratigrafico di campioni di materiale pittorico per documentare la composizione delle stratificazioni dei materiali costitutivi, dalla preparazione fino alle vernici superficiali, e avere così informazioni circa la tecnica esecutiva;
- identificazione delle cariche minerali negli strati preparatori e dei pigmenti nelle stesure pittoriche;
- riconoscimento, mediante test microchimici ed istochimici, delle classi di appartenenza dei leganti organici nelle varie stratificazioni;
- identificazione dei leganti pittorici mediante gascromatografia abbinata alla spettrometria di massa (GC-MS).

Una prima ricognizione delle superfici pittoriche delle quattro opere è stata effettuata mediante l'impiego di uno stereo-microscopio operativo con sorgenti di luce visibile e UV. Successivamente, sono state analizzate le superfici pittoriche mediante uno spettrofotometro portatile a raggi X (XRF). Sui dipinti sono state, inoltre, eseguite indagini radiografiche e riflettografiche all'IR per studiarne lo stato di conservazione e esaminare i dettagliati disegni preparatori. Una volta acquisite in situ tutte queste informazioni sono stati effettuati sedici microprelievi di materiale pittorico in zone ritenute maggiormente significative in relazione alle domande di conoscenza.

I test microanalitici impiegati sono stati finalizzati alla ricerca di sostanze proteiche, composti saponificabili e polisaccaridi. Le analisi rivolte all'identificazione dei leganti organici sono state condotte mediante microspettroscopia infrarossa a trasformate di Fourier e gascromatografia accoppiata alla spettrometria di massa (GC/MS).

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Towards the Non-invasive Proteomic Analysis of Cultural Heritage and Archaeological Objects

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During the last years, there has been a growing interest in the identification of proteinaceous material of cultural heritage and archaeological objects. Our group recently developed a new method for the non-invasive analysis of proteins material from precious and ancient artworks. The technique uses a functionalized film that extracts the proteins from the surface of the object. The extracted proteins are then analyzed by LC-MS/MS analysis using shotgun proteomics[1].

In this research, we will present the application of this method to several historical and archaeological samples. Moreover, we will discuss the analytical performances for the identification and quantification of proteins in complex matrixes.

In particular, we will present the developed protocol and the results obtained from the analysis of several artworks: a polychrome wooden altarpiece from the Maestro of Oropa, a polychrome sandstone capital, a rare polychrome alabaster, several wood medieval and renaissance panels (Pietro Gallo from Alba, Defendente Ferrari, Martino Spanzotti, Jean Bapteur, Gerolamo Giovenone) and a detached fresco from Antoine de Lonhy.

Moreover, through the use of this method we were able to identify the animal origin of an old precious manuscript from Domenico della Rovere (XIV century) as well as the binders from a rare sample of painted leather casket from Parisian manufactory (beginning of XIV century).

The analysis of the bottom of several greek vessels (Pyxis, Krater, Kylix cup and Kantharos) revealed the presence of animal origin proteins.

In conclusion, this study will report the first use of this non-invasive method for the characterization of proteins from ancient objects without the need to transport or sampling the artifacts.

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Provenance of Bitumen from Different Apulian Bronze Age Settlements Through a Biomarker Based Approach

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Bitumen has been used frequently in the Near East throughout history until modern times. Natural bitumen, found in solid or liquid form, was exploited and processed by ancient populations in Egypt, Mesopotamia and Syria for several uses such as to repair broken pottery, to waterproof containers or boats, to build the massive ziggurat temple mounds or to haft composite flint tools[1,2,3].

Together with the more widely studied and archaeologically important bitumen deposits in the Near East and Middle East[4,5], there are several seepages in Central Mediterranean which deserve more attention with respect to their exploitation and circulation in Antiquity. Evidence of bitumen use was found at several Apulian Bronze Age coastal sites[6], such as Otranto, Roca, Le Pazze (Lecce), Scoglio del Tonno (Taranto), Monopoli (Bari) and Torre Santa Sabina (Brindisi), but there are no archaeometric data about the relevant source(s).

Different bitumens from the central mediterranean sea region were studied as a possible source and analysed together with the archaeological samples using a geochemical, biomarker-based approach. The compositions of geological and archaeological samples were determined and provenance established. In the present communication, the relevant results will be described.
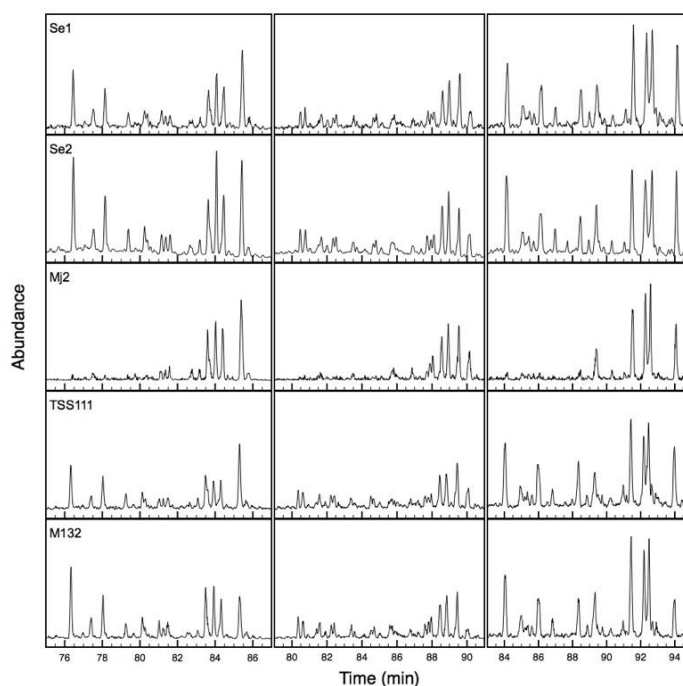
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From XAES signals to depth-profile reconstruction: the case of copper and copper alloys

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The analysis of the chemical state of co-existing copper and Cu (I) compounds by XPS is difficult since the Cu2p signal does not show any chemical shift. On the contrary the XAES signal $CuL_3M_{45}M_{45}$ allows distinguishing between Cu (0) and Cu (I) compounds. An analytical method starting from curve fitting of composed Auger induced CuLMM signals based on standards that permits quantifying the amount of copper and Cu_2O on the surface of brasses was developed and the thickness of the contamination and oxide nanolayers on the brass samples was determined by applying the three-layer model to standard XPS signals[1]. An alternative non-destructive way to reconstruct the in-depth composition profile of a nano-layered surface by XPS involves the acquisition of angle resolved XPS spectra. An ARXPS experiment provides information on the intensities of the photoelectron signals vs emission angle: the higher is the emission angle, the more intense are the signals of the elements in the most external layer. To transform this ARXPS information in concentration vs depth, an iterative protocol based on the maximum entropy method (MEM)[2] can be applied. In this work the depth profile of a S-functionalized copper sample, before and after rubbing in a tribometer was reconstructed applying MEM. The functionalization of copper with sulfur lead to the formation of Cu(I) – S bonds. The curve fitting of CuLMM (Figure 1a) allowed quantifying the % of Cu2p due to metallic copper and to Cu (I) in order to obtain the apparent concentration graph, which is the input for the MEM routine. The in-depth distribution obtained is shown in Figure 1b.
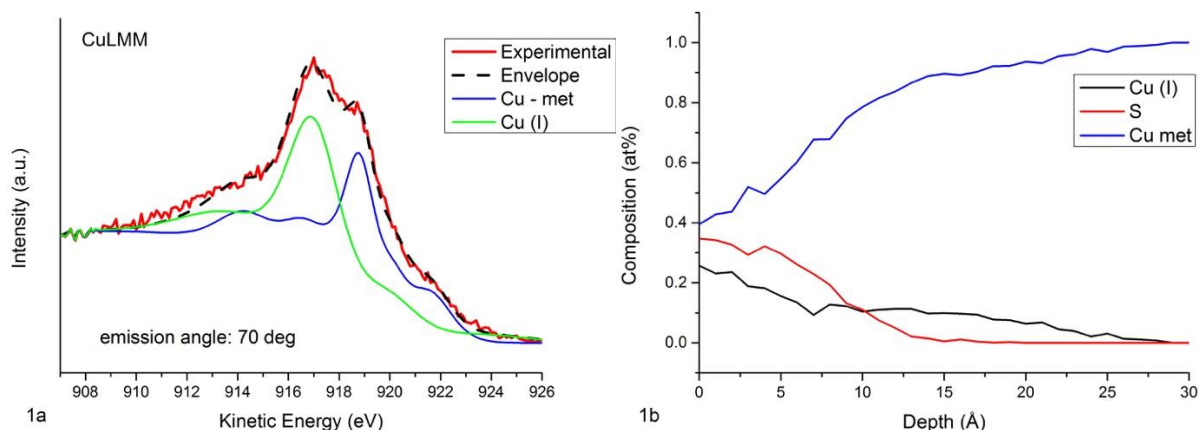
Results obtained also on the in-depth reconstruction of the oxide layer on the surface of brass alloys aged in buffer phosphate solution at pH 7 will be presented and discussed.

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Integrated Approach for the Chemical Characterization of Archaeological Woods

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Wet archaeological wooden artefacts represent a conservation challenge still far to be solved, especially when archaeological wet wood has been previously treated with unstable materials and/or contain unstable inorganic salts which influence the degradation pathways. In order to evaluate the state of conservation of archaeological wet wood we have developed since 2007 an integrated analytical approach based on NMR spectroscopy, Py-GC/MS, and GPC analysis and applied on woods from the Site of the Ancient Ships of San Rossore[1,2,3](Pisa, Italy) and Vasa[4]. Recently we have improved the analytical approach by dissolving archaeological wood into ionic liquids (ILs) as non-derivatizing solvents: in fact, 1-allyl-3-methylimidazolium chloride ([amim]Cl) can provide a homogeneous reaction medium for derivatization of wood-based lignocellulosic materials. As a result, the functionalised wood developed an enhanced solubility in molecular solvents, thus enabling information about modifications of lignin, depolymerisation of cellulose and structure of lignin-carbohydrate complexes to be obtained by means of spectroscopic (2D-HSQC-NMR and ^{31}P-NMR) and chromatographic (Gel Permeation Chromatography) techniques. Py-GC-MS was used to investigate the degradation undergone by the lignocellulosic components on the basis of their pyrolysis products, without any pre-treatment of the samples [Figure 1]. The application of all these combined techniques provided a comprehensive characterisation of the whole cell wall of archaeological wood and the evaluation of its state of preservation. These analytical techniques were applied in an integrated way for the first time on archaeological wood from the Oseberg collection. High depletion of carbohydrates and high extent of lignin oxidation were highlighted in the alum-treated objects, whereas a good preservation state was found for the untreated wood of the Oseberg ship (Norway)[5].
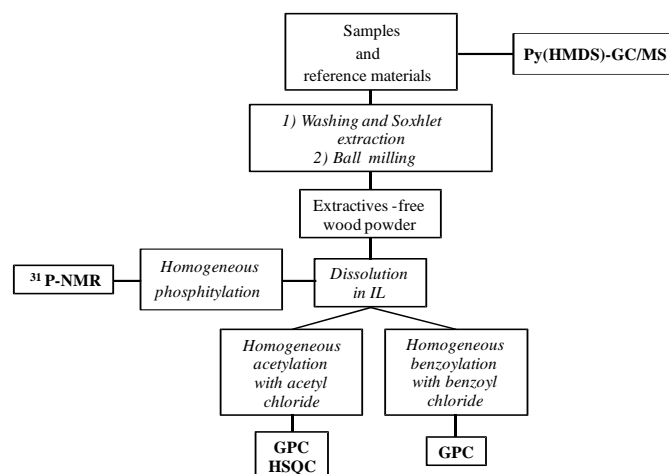
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Rapid, Low-cost and Portable Electrochemical Assay for Heart Injury Diagnosis

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Early and fast diagnosis and prognosis of heart injury are of paramount importance for a successful treatment of the diseases. Furthermore, heart related pathologies, also caused by anticancer treatment, are determinant in cancer patient survival. Several molecular markers of heart damage are emerging and electrochemically-driven trapping and signal transduction of biomarkers have proven to be effectively employed in the development of sensing devices and assays.

For instance, cytochrome c (cyt c), an inner mitochondrial membrane (IMM) protein, which plays an important role in oxidative phosphorylation, can be detected as heart failure marker. This protein is not detectable in healthy patients' blood but it has been demonstrated that during some diseases, which also involves the cellular apoptosis, cyt c is released[1]. In particular, cyt c is released from cellular membrane as a consequence of the treatment of infarction[2]. A fast monitoring of cyt c concentration can help to control the progression and extension of the heart injury guiding the therapeutic actions.

In this view, there is an urgent need to develop biochips/sensors/devices for the rapid detection of markers, such as cyt c, in very early stages of the diseases to evaluate the real state of the injury and to operate with fast bedside therapies.

Herein, we show some strategies based on optimized chemical affinity for the trapping and the detection of heart injury markers and for signal amplification.

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Carbon Black Modified Screen-Printed Electrodes to Detect Chlorine Dioxide

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Chlorine compounds have always been the most used chemical agents for water disinfection. However, alternative disinfectants have been investigated to avoid harmful by-products generated by the reaction between free chlorine and organic substances present in water. Among the different compounds used for the treatment of water, chlorine dioxide can be considered a good alternative to chlorine. Chlorine dioxide is employed in several industrial fields, including food, textile, and paper industries. Furthermore, chlorine dioxide is used to produce disinfected water with satisfactory characteristics in terms of odor and taste[1,2].

Chlorine dioxide is often used because of its excellent oxidizing and disinfecting properties against bacteria, viruses and algae, combined with the absence of harmful by-products, such as trialomethanes. Moreover, it is active in a wide range of pH, unlike free chlorine[3].

Actually, the maximum residual disinfectant level using chlorine dioxide in water is 0.8 ppm, accordingly to the United States Environmental Protection Agency (EPA), thus analytical tools are required to control the disinfectant level[4].

Due to the industrial needs in the control of disinfectant level in different processes, the development of cost-effective and easy to use sensors is highly requested.

The goal of the Tecnosens Spin-off is to fabricate miniaturized and sustainable electrochemical sensors for the monitoring of disinfectant compounds.

Herein, we report a novel and cost-effective probe for the *in-situ* determination of chlorine dioxide in swimming pool water.

This sensor was fabricated using screen-printed electrodes modified with carbon black (CB), a cost effective and raw nanomaterial with excellent electrocatalytic properties as demonstrated by our research group and corroborated by also other groups, like Compton's group[5,6].

The working electrode was modified by drop casting with 2 µl of a carbon black dispersion (1 mg/ml) prepared in a mixture of N,N-Dimethylformamide/H_2O 1:1 (v/v). Under optimized conditions (potential of + 0.1 V vs Ag/AgCl and Britton-Robinson buffer 0.02 M + KCl 0.02 M, pH =2 as working solution), the sensor showed a linear response up to 10 ppm (R=0.989), sensitivity and LOD equal to 13.4 nA/ppm and 0.03 ppm, respectively.

The interfering species including NO_3^-, SO_4^{2-}, CO_3^{2-}, HCO_3^- and Cl^- were evaluated, demonstrating the selectivity of this device.

The study of matrix effect and the accuracy of the sensor was estimated in swimming pool water, obtaining a satisfactory recovery value $(78 \pm 8)\%$.

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Bacillus Anthracis Spores Detection By Using A Label Free Impedimetric Aptasensor

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Since 1930, biological weapons have been developed and used in different countries; they include microorganisms like virus and bacteria which can be used in terroristic attacks, affecting the human health. For instance, *Salmonella typhimurium* was spread in food samples by a religious sect in the western USA; *Bacillus anthracis* spores were used in 2001 to contaminate letters in the Washington area[1].

For the safety of citizens at worldwide level, there has been a continuous interest in the development of robust and reliable analytical tools for the detection of these agents, to provide an early alarm in case of terroristic attacks. The detection of these agents is usually carried out using laboratory-set techniques like real-time PCR, which are not suitable for in field applications and fast early warning, due to the lack of portability, power requirements, long response time and expensive procedures. In this context, electrochemical biosensors offer advantages in terms of high sensitivity, miniaturization, integration, low cost, and power requirements. For the development of biosensors able to detect these agents, antibodies are mainly used as biocomponents, but their production requires the use of animals; in addition, their stability is often affected by environmental conditions. To overcome these drawbacks, synthetic DNA aptamer sequences were recently employed to develop aptasensors[2].

Herein we describe the development of an impedimetric label free biosensor for the detection of *B. anthracis* spores, using the BAS-6R aptamer as biocomponent. This aptamer sequence has been selected among a library of 92 DNA aptamers by Bruno et al., demonstrating a good selectivity for *Bacillus* spores when tested in fluorescence, using a fluorophore and a quencher as labels[3]. We developed a miniaturized label free aptasensor for *B. anthracis* spores using this aptamer as biocomponent and the electrochemical impedance spectroscopy as technique. The aptamer BAS-6R was immobilised on gold screen-printed electrodes by exploiting the affinity between the gold working electrode area and the thiol chain at the 5′ position of BAS-6R, while the spores presence has been detected by evaluating the increase of the charge transfer resistance (R_{ct}). To optimise the aptasensor, different working conditions, including the amount of aptamer and blocking agent, the time of aptamer incubation, the type of working buffer have been studied. This aptasensor was then tested against *B. anthracis* spore simulants, namely *Bacillus Cereus 14579*, *Bacillus Subtilis*, *Bacillus Cereus* 11778, observing a significant R_{ct} variation in the presence of *B. Cereus 14579* (10^5 CFU/mL), thus demonstrating the binding between the aptamer and this *Bacillus*. This aptasensor has showed the capability to detect *B. anthracis* spores simulant in the range comprised between 0.5 x 10^5 CFU/mL and 7 x 10^5 CFU/mL, it has also demonstrated its suitability for a rapid analysis.

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A Reagent-Free Paper-Based Biosensor For The Detection Of Mustard Agents

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Mustard Agents (MAs) are among the most dangerous Chemical Weapon Agents (CWAs) used during the last wars because they cause severe toxic effects on living organisms as typical skin lesions similar to burns. They embrace sulphur mustards, such as Yperite (bis(2-chloroethyl)sulphide), and nitrogen mustards, such as HN1 (bis(2-chloroethyl)ethylamine), HN2 or Mustine (bis(2-chloroethyl)methylamine), and organic arsenical Lewisite (dichloro(2-chlorovinyl)-arsine). Such kind of CWAs still represent a potential threat within the current international scenario, because of the concrete possibility that they could be used for terrorist attacks. Indeed, the Organization for the Prohibition of Chemical Weapons (OPCW) has accused Isis to have used MAs in Syria and Iraq, while ONU discovered a CWA factory nearby the city of Ninive, with several cases of people contaminated by MAs after the battle to liberate Mosul.

Classical analytical techniques such as Gas Chromatography and Liquid Chromatography are usually used for the analysis of these compounds. Electrochemical sensors represent an interesting alternative, since they can be tailor-made to improve the sensitivity and the selectivity. As they can be associated with portable, easy-to-use, and cost-effective instrumentation, they could result in user-friendly and fast alarm devices particularly suitable in case of chemical attack as well as screening tools for contaminated areas.

Recently, we have developed an amperometric bioassay for MAs detection by means of a polyester-based screen-printed electrode (SPE) modified with Prussian Blue nanoparticles[1]. Herein, we present the preliminary results obtained for MAs detection by using a filter paper-SPE, printed with a graphite-based ink containing a hybrid nanocomposite of Carbon Black and Prussian Blue nanoparticles (CBPBNPs). The use of paper as a support, further reduces the cost of the sensor, facilitates its disposal and paves the way for the construction and assembling of a reagent-free sensor. MAs simulant bis(2-chloroethyl)amine was detected by exploiting its inhibition activity toward the choline oxidase enzyme (ChOx). Choline (Ch) was used as a substrate to monitor the enzymatic activity, through the measurement of the by-product hydrogen peroxide at an applied potential of 0 mV vs Ag/AgCl. Hence, the analytical performances of CBPBNPs-based paper-SPE for hydrogen peroxide detection were investigated. The known properties of CBPBNPs to electrocatalyse the hydrogen peroxide reduction[2] allowed to enhance the analytical performances of the paper-SPE, obtaining a linear range between 0.1 and 10 mM, with a sensitivity of 3.03 $\mu AmM^{-1}cm^{-2}$. Then, the enzymatic activity of choline oxidase toward choline was studied in the range of 0.1 and 20 mM, obtaining an apparent K_M of 6.1 ± 0.5 mM. The inhibition of ChOx was thus observed by using bis(2-chloroethyl)amine from 1 to 25 mM, resulting in a linear range up to 7.5 mM, with $IC_{50} = 5.4$ mM and a LOD equal to 0.4 mM. Furthermore, the possibility to realize a reagent-free detection system was explored by loading Ch and ChOx on an origami-inspired sensor, in order to obtain a ready-to-use amperometric device for the fast and selective analysis of MAs.

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Development Of A Suitable Sequential Extraction Procedure For The Assessment Of The Availability And Reactivity Of Metal Species In Arctic PM$_{10}$ Samples

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Most of the studies on the atmospheric particulate matter (PM) trace metal content consist of the determination of the total concentrations, without distinguishing between the various chemical forms of each element. This procedure does not give any information on the different sources of PM and often provides an overestimate of potential metal toxicity, as only the soluble fraction is likely to interact with the environment and living beings. Unfortunately, the chemical complexity of PM, the extremely small particle sizes and the small quantity of sample typically collected can pose significant problems for metal speciation. In many cases the exact distribution of an element among well-defined chemical species is impossible to determine, due to the large numbers of individual species and to the lack of a universal analytical technique able to both identify and quantify all of them. Alternatively, fractionation procedures are often applied, and the classification of analytes is made according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties. This kind of classification is a valid instrument for both the identification of emission sources[1] and the evaluation of health and environmental impact[2].

Many researchers have worked on the design of extraction schemes for the sequential solubilization of metals from sediments, leading to the development of the commonly accepted three-steps BCR protocol. The adaptation of this extracting scheme to PM samples led to the development of two-steps procedures, considered a good compromise among costs, analytical times and achievable information. However, different extracting solutions, different extraction methods (ultrasounds, microwave, stirring, prolonged contact) and different techniques for the separation of the extract from the solid residue (filtration, centrifugation) are used, thus reducing the possibility to compare the results[3].

In this study the most common extracting solutions for PM fractionation have been compared: i) deionized water, due to its high representativeness of the natural solubility processes taking place in the environment; ii) diluted acetic acid, also reported in the BCR fractionation protocol, allowing a good control of the external factors that could influence the extraction and iii) a buffer solution, (ammonium acetate buffer, pH 4.5), able to maintain a good pH control throughout the extraction; the selected pH is very similar to the PM spontaneous one, in order to mime the interactions naturally occurring in the environment[4]. A two-step sequential extraction scheme was optimized by using three Certificate Reference Materials (BCR176 "City waste incineration ash", NIST1648a "Urban particulate matter" and BCR701 "Lake sediment") and comparing the results obtained applying different extraction methods and different extracting solutions. The optimized procedure was then applied to some Arctic PM$_{10}$ samples.

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DNA-based single step fluorescence detection of Domoic Acid in marine water

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Domoic Acid is a neurotoxin produced by various species of red microalgae and diatoms during the harmful algal bloom. This potent toxin affects human health through the consumption of contaminated shellfish and finfish or through water or aerosol exposure. Therefore, early detection of algal toxins is an important aspect for public safety and natural environment[1,2].

Here we have developed the first homogeneous competitive fluorescence DNA-based assay for the detection of Domoic Acid. Specifically, this assay is based on the competition between Domoic Acid conjugated with two different DNA strands and free Domoic Acid for the binding of anti-Domoic Acid antibodies. By employing this strategy, we are able to achieve single-step, label free detection of Domoic Acid in marine water without sample pre-treatment at very satisfactory level of concentrations.

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Development of an analytical strategy for the purification and identification of bioactive peptides from microalgae

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Food components have demonstrated biological activities that are being applied in the prevention, management and treatment of health conditions, including cardiovascular disease. The use of food protein-derived bioactive peptides as functional ingredients in foods is a rapidly developing area of food innovation. Bioactive peptides are short-chain proteinogenic amino acid residues joined by peptide bonds, and are produced by enzymatic cleavage of proteins during food digestion, microbial fermentation, food processing, or exogenous enzymatic hydrolysis. The interest in the functional food application of peptides is due to their demonstrated biological activities relevant to the management of human health conditions such as hypertension, oxidative stress, cancer, diabetes, inflammation, and immune disorders. Therefore, there is a need to identify sustainable protein sources and optimize technology for their isolation for niche applications. One area of current focus is the valorization of protein-rich secondary products of the agri-food industry, especially dairy whey, oilseed and fish processing meals. Moreover, marine microalgae have been identified as attractive and sustainable protein sources for industry-scale production of bioactive peptides[1]. Generally, microalgae contain 40-70% proteins, 12-30% carbohydrates, 4-20% lipids, 8-14% carotene and substantial amounts of vitamins B1, B2, B3, B6, B12, E, K, and D [2]. Importantly, microalgae proteins contain well-balanced amino acid profiles, comparable to those of egg, soybean and the FAO/WHO [3] reference pattern; however, microalgae proteins may have lower biological values, digestibility, net protein utilization and protein efficiency ratio than the gold standard, casein, and egg[2]. Microalgae synthesize all 20 proteinogenic amino acids and can be unconventional sources of essential amino acids for human nutrition. Nowadays, there are numerous commercial applications of microalgae. For example, (i) microalgae can be used to enhance the nutritional value of food and animal feed owing to their chemical composition, (ii) they play a crucial role in aquaculture and (iii) they can be incorporated into cosmetics[4].

The aim of this study was the development of an analytical strategy for the production of purified bioactive peptides from microalgae proteins, by testing 7 different extraction protocols and screening different enzymes for protein hydrolysis. The purification of peptides was carried out by multidimensional liquid chromatography. The resulting fractions were tested for antihypertensive and antioxidative activities. The most active ones were characterized by nano-liquid chromatography-tandem mass spectrometry and identified by database search. The identified peptides were further mined by in-silico analysis using PeptideRanker to ascribe a bioactivity rank to each peptide. Thus, potential bioactive peptides were synthesized and validated checking their retention times and fragmentation patterns for consistency.

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Investigation of mass transfer phenomena in new core-shell and sub-2μm fully porous chiral stationary phases for ultrafast high-performance enantioseparations

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During the last decade, the field of chiral separations experienced a radical transformation. The attention of scientists and manufacturers shifted from the research of novel chiral stationary phases (CSPs) characterized by enhanced enantioselectivity to the preparation of new CSPs suitable for high-efficient ultrafast (less than 1 minute) enantioseparations. To this purpose, kinetically high efficient particle formats, such as sub-2μm fully porous particles (FPPs) and superficially porous ones (SPPs), have been introduced in chiral chromatography and functionalized with already known chiral selectors. However, the transition from traditional enantioseparations (usually performed on 3-5 μm FPPs) to ultrafast ones has been slowed down by the lack of complete understanding of the complex mass transfer phenomena in chiral chromatography and by the practical difficulties in functionalizing small particles with chiral selectors.[1]

In this work, a Pirkle-type chiral selector (Whelk-O1) was used to functionalize both SPPs and FPPs of different diameter (including also sub-2μm fully porous ones). The CSPs were slurry packed at high pressure into columns of different geometries. The kinetic performance of these columns was evaluated in normal phase conditions for the separation of the two *trans*-stilbene oxide (TSO) enantiomers[2].

The results of peak parking experiments were interpreted in the light of a proper model of diffusion in porous media (the so-called time-averaged or parallel model was used)[3] and each contribution to band broadening was individually determined. The results shown that eddy dispersion and adsorption-desorption kinetics are the most important contributions that control the efficiency of these columns.

Additionally, to further assess the potential of these new CSPs for ultrafast enantioseparations, short columns (10 mm long) were packed with both sub-2μm and core-shell Whelk-O1 CSPs and operated at a very high flow rate (8 mL/min) to perform the separation of TSO enantiomers in less than one second[2].

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Integration of separation-based analytical platforms in the development of nanomaterials as bioactive products

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Silver nanoparticles are a promising tool to control bacterial infections, and their production and optimization is an expanding field. However, the controlled production of particles with specific properties clashes with the lack of suitable techniques to provide characterization of the newly-produced materials. This research focused on the development of a suitable analytical platform able to achieve a multiparametric assessment of nanoparticle suspensions, and its integration in the development of bioactive nanoproducts in a safety-by-design approach.
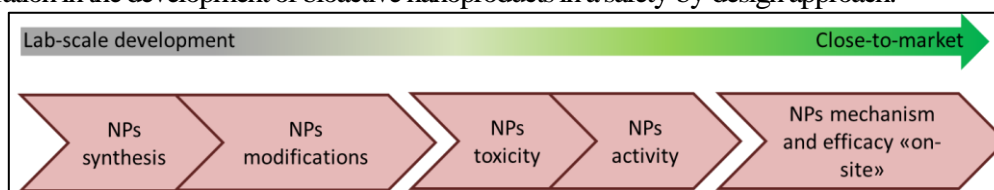
The analytical platform designed was applied in the various steps of nanoparticles synthesis and application, to provide reliable information and correlate nanoparticles properties and effects.

The separation technique used for this task was Hollow-fiber flow field-flow fractionation (HF5) coupled with UV, Multi angle light scattering (MALS) and atomic absorption detectors. The particles are characterized, separated from the unreacted media and the ionic fraction, collected and stored. Different silver nanoparticles have been tested to correlate the particles antiseptic activity and toxicity to their physicochemical properties, differing for coating and ionic content. These preparations were characterized in terms of size, shape, charge and free Ag+ amount [1].

Safety aspects were addressed by studying toxicity, inflammatory response and cellular recovery upon exposure of skin models to Ag NPs. The real time effects of the AgNPs on bacteria were also evaluated. The results obtained show that nanoparticles' activity depends not only on ionic/nano Ag ratio but also on size and surface charge of the particle[2]. An interesting effect of the positively coated Ag NPs has been observed even though the ionic/nano silver ratio is the lowest amongst all the materials tested. More importantly, these particles –though weakly toxic for human skin- remained active for greater than 7 days and remained micro-biocidal on re-challenge with freshly inoculated bacterial. By rationalizing the property-effect relationships between the evaluated parameters it was possible to categorize the key properties needed to predict nanoparticle activity, and extrapolate which combination of physicochemical properties is recommended for a successful antiseptic medical device.Then, to investigate the "nano" related risk and toxicity mechanism of silver nanoparticles and to extend this study to their close-to-market application, optimized nanoparticles were tested for NP release upon wetting/wear off. The samples collected were compared with FFF-isolated AgNPs: This approach proved to be effective and able to distinguish amongst candidates, providing a useful tool for the fast screening of nano-sized coating agents.

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Coumarin-3-carboxylic acid as ligand: acid–base properties, interactions with Fe^{3+} and characterization of complexes in aqueous solution

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The aim of this work was to determine the sequestering ability of cumarin-3-carboxylic acid (Scheme 1, generally HCCA) towards iron (III) under the physiological conditions (*i.e.* 37° C and 0.16 M NaCl).
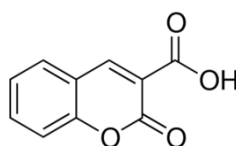
Scheme 1: Structure of HCCA.

The choice of the metal was related to the role that it plays in the biological systems and to its significant concentration in the body. Coumarins represent a new class of natural medicines potentially useful for the treatment of many diseases[1-6]. Many studies have been shown that the binding of a drug to a metal cation increases its activity, and the complex possesses much more therapeutical properties than parent drug.

The thermodynamic approach provides, first of all, the determination of the solubility and of the acidic constants of ligand under the selected experimental conditions. Then the formation constants of metal/ligand complexes were determined evaluating the competition between the protons and the iron towards ligand. The study was conducted by measuring with a glass electrode the free hydrogenation concentration in solutions containing different metal and ligand concentrations and different metal/ligand ratios, in order to determine the complexes and the corresponding stability constants $\beta_{p,q,r}$.

The investigated equilibrium can be expressed according to the following general equation:

$$p\ Fe^{3+} + r\ HCCA \rightleftarrows Fe_p(H)_{-q}(HCCA)_r^{(3p-q)+} + q\ H^+ \qquad \log \beta_{p,q,r} \pm 3\sigma$$

The characterization of the complexes was carried out with the aid of UV-Vis spectroscopy and by ICP-MS analysis.

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Ion pair formation between tertiary aliphatic amines and perchlorate in the biphasic H_2O / CH_2Cl_2 system

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The ability of selected alyphatic amines, namely tripropylamine (TPrA), trisobutylamine (TisoBuA), and tributylamine (TBuA), to form ion pairs with perchlorate anion in biphasic aqueous/dichloromethane mixtures has been demonstrated by GC with flame ionization and mass detectors, and by NMR measurements. Aliphatic amines are not present in the organic phase when the aqueous solution is at acidic pH and no perchlorate is added, whereas they are transferred with high efficiency to the dichloromethane phase when the aqueous solution is added with perchlorate 0.1 M. The extraction efficiency was modeled by theoretical equations, obtained from the mass balances and mass action laws, which were used to fit the experimental GC data. The fitting procedures allowed to determine values for K_P (partition constant of the free alyphatic amines), K^{IP} (formation constant of the ion pairs), and K_P^{IP} (partition constant of the ion pair), for TPrA, TisoBuA, TBuA at 25 °C. NMR spectra in dichloromethane before and after partition showed the presence of R_3NH^+ in the organic phase after partition, and no evidences of such species before partition. Ion pairs were shown to form in dichloromethane also when perchlorate anion is replaced by other common monocharged inorganic anions such as nitrate, chloride, chlorate, dihydrogenophosphate, and iodate. The extracted fraction of the ion pair by these ions was however lower than that observed for perchlorate. No ion pairs formed when dichloromethane is replaced by n-hexane, suggesting that aliphatic amine ion pairs can form in polar organic solvents but not in non-polar ones. The ion pair formation affects the distribution of aliphatic amines between organic and aqueous phase, and it must therefore be considered when partition coefficients and solubility data are computed for these compounds in biphasic aqueous/organic mixtures.

Acid-base and chelating properties of Gantrez copolymers

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The Gantrez (GTZ) copolymers (GTZ AN169, GTZ S97 and GTZS95) are poly(methyl vinyl ether co-maleic acid) at high molecular weight (2000, 1200 and 220 kDa), in anhydride (AN) and acid (S) form, with important characteristics (high chelating power, excellent dispersion and antisoil redeposition properties) that allow to employ them in several applications: in cosmetic, medical and industrial products. The GTZs can be considered to be a good model molecule for the study of more complex natural macromolecules, such as natural organic matter, owing to rich in carboxylic groups, which acts as a binding site for organic and inorganic cations over a wide pH range. The GTZs, when dissolved in water, form sticky and jelly solutions due to the formation of micelles in solution. The determination of the critical micelle concentration (CMC) was determined by different techniques: light scattering, conductivity, surface tension and DOSY NMR [1]. The acid-base behavior of the GTZs were studied take into account the CMC, in widely range of temperature, ionic strength and different ionic media. In the calculation of the protonation constants, a simplified approach, the diprotic-like model, was used; the model allows to treat, from acid−base and complexing points of view, a polyelectrolyte like a simple low molecular weight ligand, considering a minimum number of protonation sites necessary to extensively depict the system and in the case of the GTZs, two methyl vinyl ether co-maleic units were considered.

From the analysis of the experimental data of GTZ AN169, S97 and S95, in the same experimental conditions of temperature, ionic strength and ionic medium, it was possible to observe a trend in the protonation constants, namely an increase of the basicity of the ligands, increasing the molecular weight. The binding ability of the three copolymers was studied towards two different metal cations, Zn^{2+} and Sn^{2+} in NaCl aqueous solutions at $I = 0.15$ mol dm^{-3} and $T = 298.15$ K. The speciation model of the GTZs were compared from them and sequestering ability of these polyelectrolytes was evaluated by means the empirical parameter pL_{50}.

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Coordination of a bis–histidine oligopeptide with Ga^{3+} ion in aqueous solution

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The development of radioisotopes of inorganic elements (as ^{68}Ga(III) and ^{188}Re(I)) has gained much interest in recent years for diagnostic and therapeutic application in nuclear medicine[1]. Small peptides are employed for complexing the radiometal in a biologically stable environment. They have some advantages: easy of synthesis and modification, tumor–penetrating ability and good biocompatibility[2].

Aim of the present work has been the interaction between Ga^{3+} ion with a bis–histidine oligopeptide, PHIS (Fig.1) in aqueous solutions, by the following methods: Potentiometry, Spectrofluorimetry, FT–IR and ^{1}H–NMR Spectroscopy as also by Circular Dichroism. All the measurements have been investigated at 25 °C in 0.1 M NaClO$_4$ media. Information on coordination sites are obtained with model molecule such as

Fig.1

histidine (His). By processing the experimental data, it has been possible to obtain the complexation constants reported below.

$$\text{Ga}^{3+} + \text{HHis}^+ + 2\text{H}_2\text{O} = \text{Ga(OH)}_2\text{His}^+ + 3\text{H}^+ \qquad -6.98 \pm 0.09$$
$$\text{Ga}^{3+} + \text{H}_5\text{L}^{5+} + 2\text{H}_2\text{O} = \text{Ga(OH)}_2\text{H}_3\text{L}^{4+} + 4\text{H}^+ \qquad -9.69 \pm 0.16$$

log (constant ± 3σ)
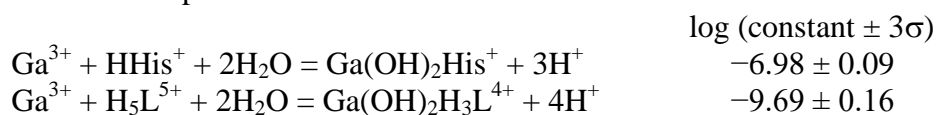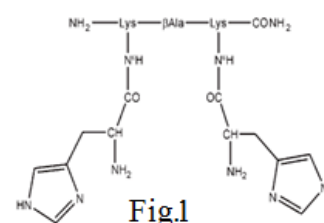
Spectroscopy data are in agreement with the potentiometric measurement. ^{1}H–NMR measurements conducted in H$_2$O as solvent at different metal/ligand ratio (0.0(A), 0.5(B), 1.0(C), 1.5(D)), indicate that imidazolic rings are involved in coordination with metal ions investigated (Fig.2). Distribution curves (in solution with 2.0×10^{-3} M in metal ion and ligand) show a wide range of pH (4–9) where Ga(III)–PHIS complex is the predominant specie (Fig.3).

Fig.2

Fig.3
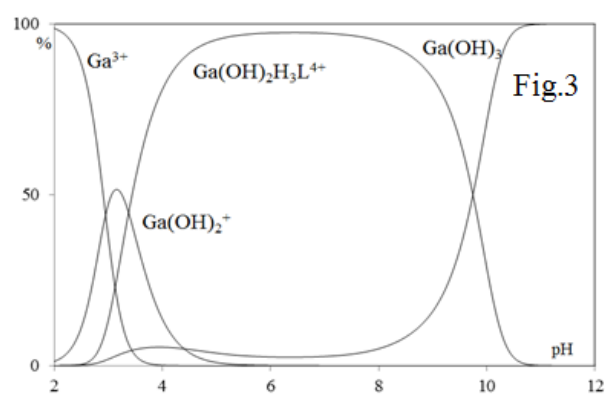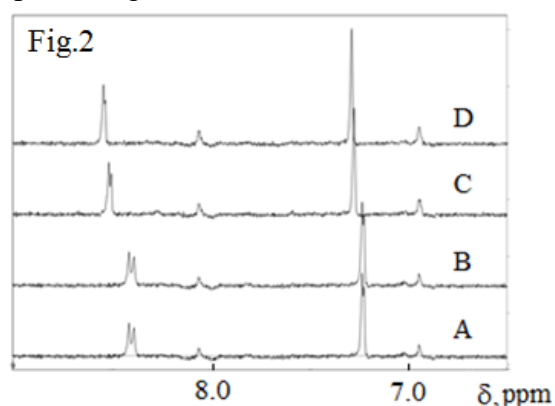
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Structural Characterization of Bio-Functionalized Gold Nanoparticles By Ultrahigh Resolution Mass Spectrometry

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Bio-functionalized gold nanoparticles (AuNPs) have a vast field of applications. The unique properties of AuNPs functionalized with biomolecules such as peptides, proteins, lipids and carbohydrates enable innovative translational research and development in biomedicine. Current research focuses for example on the development of AuNPs for imaging, photothermal therapy, vaccination strategies and drug delivery. AuNPs in the 2-100 nm size range are typically synthesized in solution by redox reactions and can be functionalized by introducing molecules containing a thiol group to form a strong nanoparticle-sulfur bond. The structural characterization of functionalized AuNPs is challenging and requires the combination of multiple analytical techniques. Mass spectrometry (MS) has been successfully used to analyze AuNPs functionalized with small synthetic ligands with molecular mass smaller than 1000 Da. Laser desorption/ionization (LDI) and matrix-assisted LDI (MALDI) have been used in combination with time-of-flight (TOF) MS to analyzed ligands directly detached from the surface of AuNPs during the ionization process. However, TOF MS provides limited performance in terms of mass resolution and MS/MS possibilities. Thus, the analysis of AuNPs ligands has been limited to the determination of molecular mass only. To overcome these limitations, we designed an new strategy for the analysis of AuNPs based on ultrahigh resolution Fourier transform ion cyclotron resonance (FTICR) MS and a combination of LDI and MALDI. Following this strategy, we comprehensively characterized the surface chemistry of AuNPs conjugated via a thiol-ending linker to either the ovalbumin peptide (OVA 323-339), the Lewis X antigen (Galβ1-4[Fucα1-3]GlcNAcβ1) trisaccharide, the tetramannoside Manα1-2Manα1-2Manα1-3Manα1, or a mixture of both carbohydrates. We analyzed all bio-functionalized AuNPs by 15T LDI/MALDI-FTICR MS (Bruker) using 1,5-diaminonaphthalene (1,5-DAN) as a MALDI matrix. We used collision-induced dissociation (CID) to characterize the structure of pseudo-molecular ions generated by LDI/MALDI, in-depth. These included $[M+H]^+$ and $[M+Na]^+$, and importantly also $[M+Au]^+$ and $[M+2Au-H]^+$ ions which provide direct evidence for the Au-conjugation of ligands. In addition, we used our strategy to monitor proteolytic cleavage of peptides conjugated to the AuNP surface.

This study presents a novel application of ultrahigh resolution LDI/MALDI-(CID)-FTICR MS for the characterization of bio-functionalized AuNPs.

Molecularly Imprinted Materials Coupled to MALDI-TOF Mass Spectrometry for the Targeted Analysis of Peptides and Proteins.

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Molecularly imprinted polymers (MIPs) are a class of tailor-made biomimetic materials, prepared by template assisted synthesis: the monomers and the crosslinker are polymerized in the presence of the target analyte, called the template, thus printing onto the growing polymeric chains both the stereo and the chemical complementarity for the template. MIPs exhibit exceptional recognition properties for the template (being this a small molecule, a peptide or even a protein) with reported affinities and selectivity of the par of natural antibodies[1-3]

With the aim at improving the analytical methodologies meant for targeted proteomics and clinical applications, we propose the development of a flexible analytical platform based on the integration of MIPs to MALDI-TOF mass spectrometry (MS) for targeted protein analysis and characterized by selective enrichment,[4] high sensitivity and selectivity and short analysis times.

Libraries of micro and nano-MIPs addressed at the recognition of peptides and proteins, including responsive MIP-materials, were synthesized by radical polymerization of acrylamido-based monomers. The prepared materials were characterized physico-chemically, showing ~2 μm microMIPs and ~50 nm nanoMIPs. The MIP compositions were confirmed by XPS analysis. The binding isotherms demonstrated nanomolar affinities for the templates. At last, the micro- and nanoMIP materials were coupled to MALDI-TOF-MS.

The analytical performance of the MIP/MALDI-TOF-MS was studied by challenging the system with selected peptides and proteins, at concentrations spanning from the nano- to the pico-molar; in model solution and in real biological specimens.[5,6]

The results demonstrated the ability of the MIP/MALDI-TOF-MS hyphenation to detect in short times (few minutes) pico- to femto-moles of the target analyte straight from serum samples, with minimal sample handling, hence proving the strength of coupling micro- and nano-MIP materials to MALDI-TOF-MS, opening up innovative analytical perspectives.
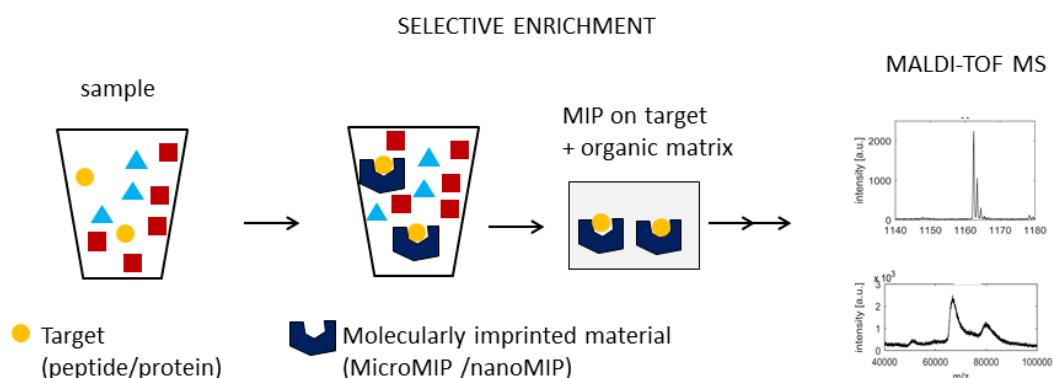
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Unknown And Non-Target Analysis To Determine Pesticides In Fruit And Vegetables By Means Of UHPLC-HRMS (Orbitrap)

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In the last years, high-resolution techniques have enhanced the number of pesticides and pesticide-related metabolites that can be detected in food. Conventionally, the detection of pesticides in food by means of both non-target and unknown screening methods has been accomplished by either GC-HRMS or LC-HRMS followed by data processing with specific, but limited, compound databases developed by companies working in the field[1]. Empirical formulas of the molecules under investigation can be generated through a combination of parameters such as mass accuracy, isotopic clusters and ion fragments in order to be used for researches on online databases (e.g. ChemSpider). This work reports a chromatographic-alignment mass-spectrometry-based approach to detect and identify pesticides and pesticide-related metabolites by comparison of matrix blank chromatograms ("CONTROL") with unknown sample chromatograms ("SAMPLE").

An UHPLC/Orbitrap system was used to carry out five chromatographic runs of both blank matrices (CONTROL) and sample matrices (SAMPLE) and a single FullScan-ddSM^2 chromatographic run.

The software calculated a SAMPLE to CONTROL *ratio* taking the average intensity of signals originated from unknown sample chromatograms and the signals originated from matrix blank chromatograms. SAMPLE/CONTROL ratios and p-values calculated on the SAMPLE signals were used to establish a threshold to filter out non-significant values. The unknown pesticides were then identified referring to online databases such as ChemSpider/Pesticides common Names, EPA Toxcast and FDA.

A specific software was used to confirm suggested compound identities and structures based on observed fragmentation patterns. All metabolites and/or degradation products of pesticides that can possibly be found in the samples can be investigated after identification through the method described above.

Quality control approach to test this method was made using SANTE/11945/2015 document as reference. A team of research unrelated to this experiment spiked samples of stone fruits with 36 different pesticides showing a wide range of physical-chemical properties thereby ensuring all compound classes detectable by LC-MS to be represented.

This method was compared to both a screening target method that uses a 350 compounds homemade database with 350 compounds and non-target method that uses a database with 650 pesticides from. The results provided an unambiguous identification and structural characterization of the compounds based on accurate mass measurement and informative fragmentation spectra resulting in no false positive and no false negative data. Moreover, this method allowed to detect and identify two metabolites undetected by both target and non-target approach.

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From Ascorbic Acid to Furan molecules: A Theoretical and Experimental study on the Gas Phase Acid Catalyzed degradation of Vitamin C

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Degradation of L-ascorbic acid (L-AA) occurs into two types of reactions, named the non-oxidative and the oxidative. The main difference between these two pathways is that furfural is more easily produced through the former. It should be noted that the expression non-oxidative refers solely to the nature of the initial step, since subsequent transformations may involve various oxidation steps. Indeed, the oxidative pathway describes the reaction which involves as an initial step the oxidation of L-ascorbic acid to dehydro-L-ascorbic acid. Likewise, the non-oxidative pathway relates to the direct decomposition of L-ascorbic acid with exclusion of dehydro-L-ascorbic acid as an intermediate structure. In 1995, furan and its derivatives were classified by the International Agency on Cancer Research (IARC) in the group 2B, as possibly carcinogenic to humans.

Since the first report in 1933, the formation of furfural from L-AA in strong acid media has been confirmed by many workers and some reaction mechanisms for the formation of furfural from L-AA have been proposed. However, none of them seems to be acceptable as the mechanism taking place in ordinary food stuffs.

Here we report on the gas-phase investigation performed by a joined mass spectrometric and theoretical approach on the acid catalysed mechanism for the formation of furan compounds in the non-oxidative degradation of L-AA. According to this approach, gaseous protonated ascorbic acid ions, $[C_6H_8O_6]H^+$, at m/z 177, were generated by Electrospray Ionization Mass Spectrometry of an ascorbic acid solution. The $[C_6H_8O_6]H^+$ ionic reactants at m/z 177 were previously structurally characterized as the ascorbic acid molecule protonated at the O2 carbonyl oxygen atom[1].

They were subjected to collisionally activated decomposition (CAD) in order to induce the gas phase unimolecular degradation pathway of protonated ascorbic acid.

The degradation pathway emerging from the CAD mass spectrum of the precursor ion at m/z 177 shows a twofold dehydration step, 177→159 and 159→141 followed by the elimination of an $HCOOH/CO_2$ moiety, 141→95/97, leading to the formation of furanic products.

Energy Resolved CAD mass spectra allowed to obtain informations on the relative energies of degradations steps. Experimental results were compared with those of a theoretical investigation performed at B3LYP/6-31+G(d,p) level of theory highlighting the most favourable decomposition pathway. The mechanism leading to furan compounds involves dehydration, hydrolysis of the lactone ring followed by decarboxylation.

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Liquid-EI (LEI) Atmospheric Pressure Mechanism for the introduction of liquid streams into an unmodified electron ionization source of a mass spectrometer.

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We have combined, for the first time, an atmospheric pressure gas-phase conversion mechanism with new ceramic coatings to create an innovative interface, called Liquid-EI (LEI) (1). LEI is based on electron ionization (EI) but differs from previous attempts; the vaporization of solutes and mobile phase takes place at atmospheric pressure into a specifically designed region, called the "vaporization micro-channel", before entering the high-vacuum ion source. The interface is completely independent from the rest of the instrumentation, and can be adapted to any gas chromatography-mass spectrometry (GC-MS) system, as an add-on for a rapid LC-MS conversion. A ceramic liner, placed inside the vaporization micro-channel, acts as an inert, 'non-stick' vaporization surface, speeding up the gas-phase conversion of large molecules while lessening possible memory effects.

EI is an unparalleled, well-established tool for the identification of unknown gas-phase molecules. Its extension to a liquid phase, without the drawbacks and limitations that troubled this hybrid combination to date, provide the same unique advantages (library searchable mass spectra, robustness, negligible matrix effects) to LC amenable compounds, opening the door to new, challenging LC-MS applications. Deactivated silica coatings help to release the heaviest compounds to the gas-phase, improving vaporization efficiency and reducing high-temperature contact time for the most labile substances, bridging the gap between the world of classic LC-MS and GC-MS.

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Selective gas-phase conversion of D-fructose to 5-hydroxymethylfuraldehyde through a base-assisted dehydration process

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5-hydroxymethylfuraldehyde (5-HMF) is the main product of the thermal acid-catalysed dehydration of monosaccharides and together with other furan compounds is considered a platform molecule for the production of chemicals and fuels. Identifying alternative catalytic strategies to synthesize 5-HMF represents a key-step to increase reaction selectivity and reduce degradation-by-product yields. In this regard, mass spectrometry has proved to be an useful tool for studying reaction mechanisms in absence of solvent molecules. This approach has already been employed to investigate the acid-catalysed D-glucose and D-fructose dehydration mechanism, highlighting the formation in the gas-phase of a 5-HMF protomers and isomers mixed population (1,2). In this work the effect produced by nitrogen-containing bases on the D-fructose dehydration reaction has been evaluated using tandem mass spectrometry. Ionic complexes formed by the protonated sugar and a nitrogen base were allowed to undergo collision-induced dissociation (CID) in an ion trap mass spectrometer. The dehydration process was followed step-by-step by isolating in turn the resulting ionic intermediates that still retain the attached bases depending on their proton affinity values. The sequential fragmentation leads to the formation of $[C_6H_6O_3]H^+$ ions corresponding to a pure protonated 5-HMF population when the base loss occurs as the last reaction event (Figure 1). This evidence demonstrates the existence of a selective and effective base-assisted mechanism. Theoretical calculations are in progress in order to: i) elucidate the structures of the starting reactant ion and of the intermediates ii) validate a feasible reaction mechanism.
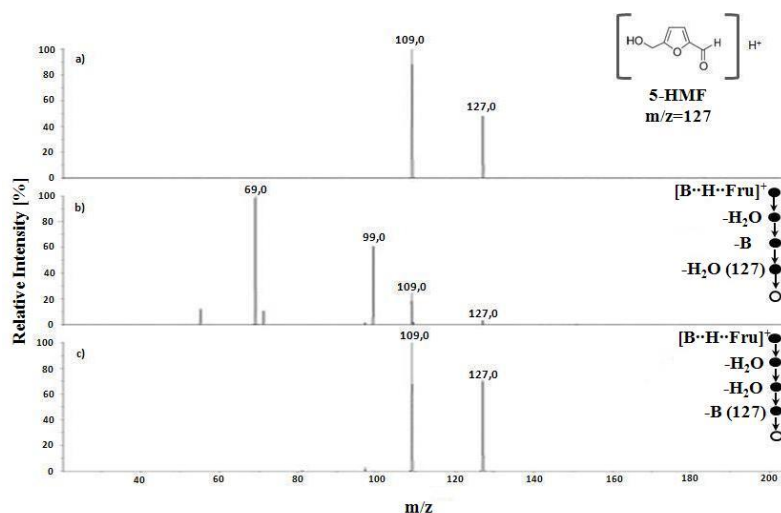
Fig. 1 CID mass spectra of a) protonated standard 5-HMF, b) ions at m/z 127 obtained after a premature base loss and c) ions at m/z 127 arising when the base loss occurs as the last reaction event.

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Development of a new platform for chemical sensors based on surface plasmon resonance and molecularly imprinted polymers as receptors

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Optical sensors based on POF have some advantages over those based on different waveguides, as for example the prism of the classical Biacore instrument. One of them, particularly relevant, is the possibility of remote sensing. A problem could be the integration of the receptor with the fiber, for which different strategies have been proposed[1]. Particularly useful seems to be a platform recently proposed by our group[2], consisting in a D-shaped plastic optical fiber without cladding, in contact with a thin gold layer. The fiber is maintained in a fixed and stable position by a small cubic holder purposely designed. The artificial receptor, i.e. the molecularly imprinted polymer, is deposited over the gold layer, so that it constitutes the dielectric medium whose refractive index determines the plasmon resonance effect. In the present investigation a new sensor platform, based on surface plasmon resonance (SPR) phenomenon (based on a slab waveguide and two POFs), is presented. In this case the waveguide for SPR is a thin chip of the same material of the optical fiber core (PMMA), not the optical fiber itself. The optical fiber is used to introduce the light into the slab waveguide, exploiting the evanescent wave phenomenon, and to translate the light emerging from the waveguide to the spectrometer (see Figure 1). A thin gold layer (60 um) is deposited over the PMMA chip (slab waveguide), and the molecularly imprinted polymer is deposited over it. This operation is relatively easy to perform, due to the flat shape of the gold surface. Other advantages of this new approach are the possibility of sensing with a removable chip, the easy production of an engineered platform and the use of a new holder, which is also suitable for thermo-stabilized flow cell in chemical sensing applications.

Figure 1. Experimental setup of the sensor system

It has been found that the performances obtained by this configuration are very similar to those obtained by the previously proposed platform (2). In particular, the optical sensitivity and the resolution are similar, being respectively 1500 nm/RIU and 10^{-3} RIU. These preliminary results showed that the sensor's performances make it suitable for chemical applications.

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Enantioselective voltammetry on achiral electrodes: a comparison between inherently chiral additives based on different stereogenic elements

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An attractive target in electroanalysis is the availability of chiral media affording enantioselection in terms of significant peak potential difference between the antipodes of chiral probes in voltammetry experiments on achiral electrodes. Previous literature attempts pointed to enantioselectivity increasing with the structural order of the chiral medium; on the other hand, outstanding enantioselection performances have been recently observed working on electrode surfaces consisting in "inherently chiral" oligomer films, that is, in which chirality does not originate from localized stereocenters, but is intrinsic of the whole main molecular backbone, featuring a tailored torsion[1-5]. Combining both strategies we have recently developed two inherently chiral ionic liquids ICILs, consisting of dialkylated bicollidinium salts, with an atropoisomeric bipyridinium cation featuring at least one octyl chain and bistrifilimide counteranions. They showed high enantioselectivity when tested even as low concentration additives in commercial achiral ionic liquid media[6]. Importantly, similar ability was also shown by other terms of the same family, having shorter alkyl chains and/or different counteranions, solid at room temperature but of easier synthesis. As a first temptative explanation we are considering the high supramolecular order of even simple ionic liquids at the interphase with a charged surface. A chiral additive could result in chiral reorganization of this peculiar interphase, as in the case of nematic-to-cholesteric transitions induced by chiral dopants in liquid crystals[6]. Such application of the chiral selector as additive in an achiral ionic liquid rather than as the bulk medium is indeed interesting and convenient, since only a small quantity of the enantiopure selector is needed and a low melting point is no more required, but only sufficient solubility in the achiral ionic liquid. This allowed us to include in our chiral voltammetry experiments a quite larger number of inherently chiral selectors based on different stereogenic elements, *i.e.*, the bicollidine and bibenzimidazole atropoisomeric scaffolds and the tetrathielicene helicoidal scaffold. They all proved successful. Large potential differences were observed on achiral electrodes for the enantiomers of chiral probes even of very different structure and electroactivity, as in the former tests on inherently chiral electrodes, and possibly even in experiments with more than one probe, providing further evidence of the general validity of the "inherent chirality" strategy in the development of efficient chiral selectors in electroanalysis.

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Hydrogen peroxide sensitive and selective biosensors based on peroxidases from different sources wired by Os-polymer

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A comparison has been made between two plant peroxidases, cationic horseradish peroxidase (HRP) and anionic tobacco peroxidase (TOP) (1), to form the basis together with a highly cationic osmium polymer [Os(4,4′-dimethyl-2,2′-bipyridine)$_2$poly(N-vinylimidazole)$_{10}$Cl]$^{+2/+}$ ([Os(dmp)PVI]$^{+/2+}$) (2) to prepare highly sensitive, stable and selective hydrogen peroxide biosensors (3). The two different plant peroxidases were individually immobilized onto graphite rod (G) electrodes by a three steps drop-casting procedure consisting of the subsequent deposition of an aqueous solution of ([Os(dmp)PVI]$^{+/2+}$), followed by a solution of poly(ethyleneglycol) diglycidyl ether (PEGDGE), used as a cross linking agent and finally an aliquot of a solution of anionic HRP or cationic TOP to make HRP/PEGDGE/[Os(dmp)PVI]$^{+/2+}$/G and TOP/PEGDGE/[Os(dmp)PVI]$^{+/2+}$/G based electrodes, respectively (4). Electrochemical experiments were carried out to investigate the influence of the surface charge of the enzyme and the charge of the polymer on the efficiency of the electron transfer (ET) between the enzyme and the wiring redox polymer and the efficiency for electrocatalytic reduction of H$_2$O$_2$. In the case of HRP a decrease in the ET rate was observed due to the repulsion between this enzyme and the polymer, both positively charged, whereas with TOP there was an enhanced ET rate due to the attraction between the anionic enzyme and the cationic polymer. The effects of enzyme loading and pH were investigated. Both optimized peroxidase modified electrodes exhibited a wide dynamic response range (1-500 μM H$_2$O$_2$) and a low detection limit (0.3 μM H$_2$O$_2$). The TOP based electrode showed a higher sensitivity (467.4 nA μM^{-1} cm^{-2}) compared to that of the HRP based electrode (297.1 nA μM^{-1} cm^{-2}) and an improved long-term stability (decrease in 17.3% upon 30 days). Both enzyme electrodes showed a response time of 3 s. The HRP based sensor was more sensitive to the presence of phenolic compounds acting as alternative electron donors whereas the TOP based sensor was virtually interference free. Both HRP and TOP based electrodes were successfully tested in contact lens cleaning samples and real "spiked" samples from different sources such as tap water, milk and dairy products.

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Seasonal variations of major phospholipids in mussels of sp. *Mytilus galloprovincialis*: an investigation by hydrophilic interaction liquid chromatography-electrospray ionization Fourier-transform mass spectrometry

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Mytilus galloprovincialis, also known as *Mediterranean mussel*, is one of the most commercially relevant species belonging to the genus *Mytilus*. With more than 500 ktons per year its production represents the top shellfish-oriented aquaculture activity in Europe (1). Since *M. galloprovincialis* is mainly commercialized as alive/fresh product, thus having a few days of shelf life, its availability on the market is naturally subjected to seasonal fluctuations, usually due to three synergic factors: breeding technique, natural recruitment of juveniles and reproductive cycle. The latter, as well as mussel biochemical composition, is known to be strongly influenced by water temperature and food availability, in turn related to season and geographical area, since mussels are a typical product of non-fed aquaculture, thus their feeding depends on the natural availability of nutrients (2). The effects of environmental temperature on the viability of cultured mussels have been dramatically confirmed by the massive loss of production, and the consequent economical damage, occurred in several Italian aquacultural plants in July 2015, because of a prolonged heat wave, finally leading sea temperatures to rise up to 35 °C. In the perspective of a worsening of environmental conditions due to global warming, the monitoring of mussel viability through appropriate molecular markers could be very useful to contain such losses in case of sea water over-heating, e.g. by moving the production to naturally colder basins, when possible. Recently, high efficiency hydrophilic interaction liquid chromatography (HILIC) and high resolution/accuracy electrospray ionization – Fourier-transform mass spectrometry (ESI-FTMS) have been exploited for the systematic characterization of *lyso*-phospholipids in mussel lipid extracts, showing their correlation with thermal stresses occurring on mussels (3). Starting from these premises an extended characterization of the two main classes of mussel phospholipids (PL), namely phosphatidylcholines (PC) and phosphatidyl-ethanolamines (PE), has been undertaken to verify how seasonal variations may affect their profile and, consequently, if lipid species can be considered indirect markers of changing sea temperatures as a result of adaptation of mussels to adverse environmental conditions. So far more than 300 different compounds, namely 185 PC and 131 PE, have been recognized, including plasma(e)nylic species, i.e. PL bearing one of the side chains linked to glycerol through an ether bond, that were the prevailing species among PE. Their characterization by HILIC-ESI-FTMS analysis will be described in the present communication. Moreover, the specific differences between PC and PE profiles of mussels collected in different seasons, emphasized by a chemometric approach, will be discussed.

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Applications of Porous Graphitic Carbon in Liquid Chromatography – Mass Spectrometry

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The remarkable improvements in hyphenated analytical methods over the last two decades have significantly broadened their applications however, in several important cases, the interfacing of separation techniques with mass spectrometry still suffer of poor compatibility. Among different approaches, porous graphitic carbon (PGC) is a HPLC stationary phase which appear especially appropriate for small ions or neutral polar compounds scarcely retained by reversed phases[1] nevertheless, in spite of its attractive peculiar characteristics, the use of porous graphitic carbon (PGC) never took off in standard environmental and bio-analytical labs. A possible reason for it lies in the complexity of its dual behavior[2]. PGC, in fact, provides a mixture of interactions[3], both strongly hydrophobic and electronic[4], thus making difficult the predictions regarding its retention ability. Another possible reason for the "underuse" of this stationary phase is that many components of environmental or biological samples can be strongly adsorbed on it suddenly modifying the retention characteristic of the column[5]. This might result in the need for an additional column washing and reconditioning procedure. Nevertheless, several challenging separations of ionic or highly polar and hydrophilic compounds can benefit of PGC retention abilities and its excellent selectivity[6,7]. The results of several studies devoted to outline general use guidelines for PGC in combination with ESI-MS detection will be presented and the performances compared with those of ion chromatography (IC), classic reversed phase (RP) and hydrophilic interaction chromatography (HILIC).

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Synthetic Receptors for Troponin T Detection by SPR Transduction for Acute Myocardial Infarction Diagnosis

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Acute Acute myocardial infarction (AMI) is a prominent cause of mortality worldwide with a very high economic impact[1]. Currently, cardiac Troponin (cTn) isoforms I and T are the preferred diagnostic biomarkers of myocyte necrosis because when the heart cells are damaged this protein is quickly released into the bloodstream and its early detection can improve the survival rate[1,2]. Among the several methods used for cardiac biomarkers detection, affinity-based biosensors appear the most promising, due to mandatory demand of fast and reliable answer upon recognition of target molecules with high specificity. In this framework, we report the outcomes of our research dealing with the development of biosensing platforms based on SPR (Surface Plasmon Resonance) transduction. In particular, the detection of the cardiac biomarker cTnT by testing emerging synthetic receptors alternative to antibodies[3], like aptamers (oligonucleotides <100mer)[4], and MIPs (Molecularly Imprinted Polymers)[5] is here presented. These biomimetic have been already successfully exploited for numerous biosensors applications, including electrochemical methods for cTnT detection[6,7,8]. Here we report for the first time their perspective use for optical-based biosensing.

For this purpose, several target recognition strategies have been designed and investigated to enhance the system performance, in terms of sensitivity, selectivity and reproducibility for this point-of-care approach with promising social and economic beneficial impact.

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Nano- and Micro-particles in Food and Consumer Products: the Role of the Field Flow Fractionation Techniques in Their Characterization

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Nanomaterials (NMs) cover a broad range of materials of different chemical composition. Because of their diverse properties, they are used in a wide range of applications and products, such as cosmetic products (e.g. UV absorbers in sunscreens), food (enhanced flavour and texture, encapsulation of micronutrients), medical devices (diagnostics, drug delivery), medicinal products.

The recent abundant use of NMs has placed, however, the accent on their potential risk, since nanoparticles, with their sizes between 1-100 nm, might interact with membrane cells of any living beings (plants, animals, humans). Consequently, legislators who are dealing with health and consumer protection have asked to the scientific community to implement or set-up new analytical methods able to detect and characterize the nanoparticles contained especially in food and consumer products to evaluate the risk on a solid definition of NMs.

This work will illustrate, as the Field Flow Fractionation techniques (FFF), might be useful separation instruments to sort complex samples[1] and to determine the average sizes and the particle size distribution of nano- and micro-particles. FFF techniques are often coupled online with a series of detectors such as UV-vis, refractive index, fluorescence, scattering detectors (DLS or MALS), ICP-MS, ICP-OES, GFAAS, whose choice depends on the practical application.

Examples of SiO_2, silver, and ZnO separations will be presented in the light of the characterization suggested by the European Commission[2].

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Evaluation and Comparison of Gel-based and Gel-free Approaches for the In-depth Exploration of Milk Rice Proteome

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Nowadays a growing number of consumers opt for plant-based milk substitutes for medical reasons (such as lactose intolerance and cow's milk allergy) or as a lifestyle choice[1]. Also, in countries where mammal milk is scarce and expensive, plant milk substitutes serve as a more affordable option. Among the plant milk there is milk extracted from rice. Rice milk is made from boiled rice (both brown and white rice versions are available), and it is often supplemented with flavour enhancers like brown rice syrup and vanilla to help it taste more like cow's milk. Interestingly, it has been reported that rice milk represents a better alternative than soy milk for infants allergic to cow's milk. However, despite the known benefits and potential of rice milk, a detailed characterization of its protein content is needed.

In this study, in order to provide the most complete characterization of the proteins contained in the rice milk we have evaluated and compared different gel based and gel free proteomic approaches. In particular, we compared the results obtained from LC-MS/MS analysis after protein precipitation with acetone or TCA, or after protein prefractionation based on SDS-PAGE (GeLC-MS/MS) or ProteoMiner™, or after prefractionation based on peptide IEF (pIEF-LC-MS/MS)[2]. We also estimated the abundance of the identified proteins by using the exponentially modified protein abundance index (emPAI). Overall, a total of 158 different protein species based on a total of 585 peptides have been identified for the first time in the rice milk. Interestingly, the results obtained show that applying peptide IEF can significantly increase the number of proteins identified from rice milk. We also demonstrated that the rice milk proteome contains proteins mainly characterized by a Mw between 10 and 70 kDa and acidic pI, with approximately 55% of the identified proteins within the range of pI 5.0–7.0. Moreover, most of the identified proteins showed a negative GRAVY index, confirming the prevalence of hydrophilic proteins in plant milk. The identified proteins were also grouped into cellular roles based to Gene Ontology annotation according to molecular function, biological process, and cellular component. In particular, catalytic activity (56%) and metabolic process (41%) are the two most representative categories of this milk proteome. In conclusion, we demonstrate that efficient fractionation of peptides by IEF is an essential prerequisite for comprehensive analysis of protein mixtures by shotgun mass spectrometry, and more specifically for a complete characterization of the milk rice proteome.

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Aromatic Amines in Textile as Potential Threat for Human Health

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Possible chemicals found in textile samples have various sources or are added with different purposes. Pesticides can for instance be used in the production step of natural fabrics[1], added as repellent during the storage[2], or applied in anti-bug clothes improving the technical characteristics[3]. However, the main chemical source in common textile samples is the colouring step when the dyes are added. Depending on the purity, remaining reaction reagents can be present as dyes impurities[4], or parts of the dye structure can be released on the human skin by azo reduction of anaerobic bacteria[5].

In vivo experiments showed that a flame retardant, impregnated in sleepwear, can migrate from the fabric to the skin and further into the human body, since its metabolite could be detected in the subject's urine samples[6]. In our previous studies on different kind of textiles consumer product several highly toxic compounds were detected[7,8]. Among them we decided to focus on benzothiazole and its derivatives since is one of the most frequently detected compound. In another study of human skin excretions, was detected in some samples from the skin surface[9]. For this reasons, in order to assess the risk of benzothiazoles migration from worn clothes into and through the human, we have made transdermal diffusion tests on a synthetic, non-animal based model membrane using Franz cells.

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A Greener Approach for Organophosphate Flame Retardant Determination in Airborne Particulate Matter: Microwave-assisted Extraction Using Hydroalcoholic Mixture Coupled with Solid Phase Microextraction Gas Chromatography Tandem Mass Spectrometry

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Organophosphate esters (OPEs) are compounds widely used as flame retardants and plasticizers in different types of materials. Due to their extensive use, they have become ubiquitous pollutants in both outdoor and indoor environments[1,2,3].

In this work, a new analytical method for OPEs quantification in airborne particulate matter (PM) was developed. The proposed protocol provides for the microwave-assisted extraction (MAE) of the analytes from the PM followed by solid phase microextraction gas chromatography tandem mass spectrometry determination (SPME-GC-MS/MS). Ten organophosphate esters were taken into account i.e., tripropyl phosphate (TPP), tri-n-butyl phosphate (TBP), tris(2-chloroethyl) phosphate (TCEP), tris(chloropropyl) phosphate (TCPP), tris(1,3-dichloro-2-propyl) phosphate (TDCPP), tributoxyethyl phosphate (TBEP), triphenyl phosphate (TPhP), 2-ethylhexyl-diphenyl phosphate (EHDPP), tris(2-ethylhexyl) phosphate (TEHP) and tricresyl phosphate (TCP). OPEs have been usually extracted from particulate matter by using traditional approaches including soxhlet[3] and ultrasonic extraction[4] with hexane, acetone or more toxic chlorinated solvents.

In the developed method, the OPEs were extracted by MAE using a green hydroalcoholic mixture composed of water and ethanol. Design of Experiment (DoE) was used for the multivariate optimization of the parameters affecting both the MAE extraction and the SPME analysis. A 2^{5-1} fractional factorial design was performed in order to determine the factors of the MAE that have a significant influence on the analyte extraction. Later on, the most important variables were further optimized by central composite design (CCD), thereby achieving the optimal working conditions. The extraction performance of five SPME fibers was evaluated. The DVB/CAR/PDMS coating demonstrated to be the most suitable for the extraction of the target analytes. The factors affecting the SPME extraction (i.e., extraction time, extraction temperature and ionic strength of the sample) were optimized by CCD. The optimal working condition determined by using Derringer's desirability function were 45 min as extraction time, 80°C as extraction temperature and 10.0% (w/w) of NaCl. The assay of the analytes was performed by using tandem mass spectrometry in selected reaction monitoring (SRM) acquisition mode. The proposed method was carefully validated. Satisfactory values were achieved in terms of linearity, accuracy, precision (Intra- and inter- day) and limit of quantification.

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Ultrasound-Vortex-Assisted Liquid-Liquid Micro-Extraction for Simultaneous Determination of Organophosphorus Pesticides and Phthalates in Baby Foods

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Baby food is any soft, easily consumed food, other than breastmilk or infant formula, that is made specifically for babies, roughly between the ages of four to six months and two years. The food comes in multiple varieties and tastes. The primary consumers of baby food, generally meets or significantly exceeds the recommended amount of macronutrients.

Baby foods are either a soft, liquid paste or an easily chewed food since babies lack developed muscles and teeth to effectively chew. Babies typically move to consuming baby food once nursing or formula is not sufficient for the child's appetite. Homemade baby food is less expensive than commercial baby foods. Homemade food is appropriate only when the family has a sufficient and varied diet, as well as access to refrigeration and basic sanitation. It is important to follow proper sanitation methods when preparing homemade baby food such as washing and rinsing vegetables or fruit, as well as the cooking and packaging materials that will be used. Some commercial baby foods have been criticized for their contents.

This communication is focused on the determination of organophosphorus pesticides (OPs) and phthalates (PAEs) by means of a method based on ultrasound-vortex-assisted liquid-liquid microextraction coupled with GC-IT/MS (UVALLME-GC-IT/MS). The developed protocol allows the determination of six PAEs (1 DMP, 2 DEP, 3 DBP, 4 iBcEP, 5 BBP, 6 DEHP; below the GC-MS chromatogram of a turkey sample is reported) and 16 OPs. 0.1 g of freeze-dried product sample are dissolved in 10 mL of warm distilled water along with 2 µL of Internal Standard (anthracene 10 mg mL^{-1} in acetone): the choice of extraction solvent is studied starting from the n-heptane, just used for PAE determination in similar matrixes. The solution, held for 5 minutes on the vortex mixer and for 6 min in an ultrasonic bath to 100 W for favoring the solvent dispersion and consequently the analyte extraction, is centrifuged at 4000 rpm for 30 minutes: 1 µL is injected into the GC-IT/MS (capillary column SE54: 30 m, ID 0.25 µm, d 0.25 mm). All the analytical parameters investigated will be deeply discussed. The method has been applied to real commercial freeze-dried samples: the results do not show significant contaminant values.
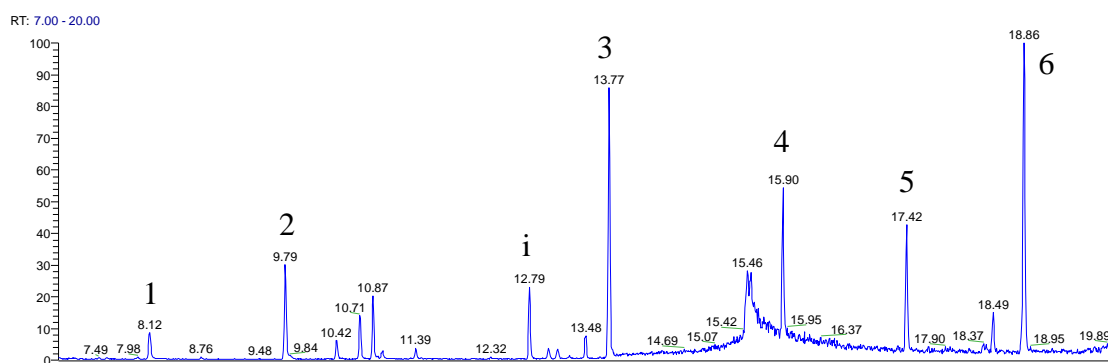
Calibration of Commercial and Modified Polar Organic Chemical Integrative Samplers (POCIS) for the Analysis of Emerging Pollutants in Waters

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Human health and ecosystems are threatened by the release and the accumulation of emerging pollutants in the aquatic environment, which needs to be constantly monitored. For this reason, many recent studies are focused on the analysis of compounds used in everyday life, like pharmaceuticals or personal care products. These substances are not entirely absorbed by human body, reaching the aquatic compartment mainly from wastewater treatment plants and effluents from hospitals and pharmaceutical production facilities[1]. The main analytical problems related to the determination of these compounds are represented by their very low concentration levels (ng/L or below) and the presence of potential interferents[2]. Therefore, the combination of passive sampling and LC-MS/MS represents an alternative and powerful approach to successfully reach the goal of their analytical determination.

Our group has developed a method for the determination of nonsteroidal anti-inflammatory drugs in drinking water by passive sampling[3], in particular using the Polar Organic Chemical Integrative Samplers (POCIS)[4]. Analyte concentration measured in the POCIS sampler can provide Time-Weighted Average concentration of contaminants in water if the sampling rate of each specific compound is known; a simple calibration system has been developed to this aim[5]. More recently, the method has been extended to other compounds which are representative of various classes of emerging pollutants: pharmaceuticals (ibuprofen, naproxen, diclofenac, ketoprofen, mefenamic acid, gemfibrozil, carbamazepine), perfluorinated compounds (PFOA, PFOS), triclosan (an antimicrobial agent used in many personal care products) and caffeine (a potential chemical marker of human contamination). Analyses were carried out on an Agilent 1200 SL Liquid Chromatograph with Zorbax XDB-C18 column, coupled to an Agilent 6430 Triple Quadrupole equipped with ESI interface; quantitation was achieved by multiple reaction monitoring mode to maximize sensitivity, using the internal standard method with isotopically labelled compounds.

The effect of different environmental condition (i.e. water flow and temperature) on the POCIS calibration has been studied. Besides, a new configuration of the sampler, which differs from the commercial one for the membrane porosity has been studied; the first results are really interesting, highlighting a significant enhancement in the sampling rate for many analytes. If this will be confirmed it should be possible to reduce the deployment time of the POCIS, extending the applications of this passive sampler.

Examples of monitoring studies investigating the presence of these emerging pollutants in drinking water treatment plants of Northern Italy will be presented.

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A New Validated Gas Chromatographic-barrier Ionization Discharge Detector (GC-BID) Method for the Monitoring of CO_2 and N_2O Emissions from Settling and Disinfection Units in Wastewater Treatment Plants (WWTPs)

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The reduction of greenhouse gas (GHG) emissions is nowadays a well-defined path at European level far beyond the fateful 20-20-20 agreed in Kyoto in order to achieve a climate change mitigation (https://ec.europa.eu/clima/policies/strategies/2050_en). In this respect, the monitoring of GHG emissions from wastewater treatment plants (WWTPs) is of increasing interest, to improve the knowledge about biological processes and operational conditions in WWTPs for lowering the environmental impact[1]. However, standard procedures are not available yet, so there is a need for analytical methods to identify and quantify these gases (i.e. CO_2 and N_2O). To our knowledge, even though a broad spectrum of detectors, chromatographic columns and operating conditions have been proposed for analyses of CO_2 and N_2O, few studies dealing with their simultaneous analysis are actually available in the scientific literature[2]. In this communication, we describe the development and the validation of a method based on gas chromatography (GC) equipped with a barrier ionization discharge (BID) detector, allowing us the simultaneous high-precision measurements of N_2O and CO_2 emissions. For CO_2 and N_2O it was possible to validate the method with relative standard deviations (RSD%) less than 6.6% and 5.1%, respectively, in agreement with the ICH acceptance criteria for precision[3]. In order to support the method's accuracy, gas standard mixtures containing mixed certified concentrations of CO_2 (542 and 730 ppm_v) and N_2O (400 and 602 ppb_v) were used as reference materials. A good linearity was achieved, with a determination coefficient (R^2) not lower than 0.998, within concentration ranges of 50-1000 ppm_v and 250-50000 ppb_v for CO_2 and N_2O, respectively. The limit of detection (LoD) was 62.0 ppb_v for N_2O and 5.3 ppm_v for CO_2; the limit of quantification (LoQ) was 188.0 ppb_v for N_2O and 16.0 ppm_v for CO_2 [4]. The validated GC-BID method was successfully applied for analysis of N_2O and CO_2 emissions from full-scale WWTP located in Potenza (Basilicata, southern Italy), focusing the attention on not-areated units (i.e., secondary settling and disinfection unit) poorly investigated[5]. The obtained results suggested that the validated GC-BID method could contribute to the further development of a standard methodology for GHGs measurement, which is actually not available. Moreover, innovative configurations of WWTPs could be proposed for settlers in order to reduce the wind effects on GHG emissions, mostly in windy areas and the alternative oxidants could be used as disinfection agents.

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Development of a Prototype Analytical Apparatus for the Quantitative Determination of the Composition of Fluids in Synthetic Rocks.

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The quantitative assessment of fluids' composition arising from rocks formation processes is crucial in modeling geological processes. The relative concentrations of the so called "COH fluids" (namely, H_2O, CO_2, CH_4, CO, H_2, O_2, together with N_2) can drive the formation process to completely different directions: as an example, the H_2O/CO_2 ratio, can influence the melting temperatures, the location of hydration or carbonation reactions, and the solute transport capability in several rock systems. In the scientific literature, COH fluids speciation has been generally assumed on the basis of thermodynamic calculations using equations of state of simple H_2O–nonpolar gas systems (e.g., H_2O–CO_2–CH_4). The limit of this approach relies on the fact that the behavior of more complex systems is predicted in a rough way. An apparatus which is able to perform the quantitative determination of COH fluids in experimental rocks synthesis is then needed to study such complex systems in a proper way.

From an analytical point of view, this is a quite intriguing challenge because, as very high pressures ($P > 1GPa$) are employed, only few milligrams of rock can be prepared, with fluids representing about 10^{-3}-10^{-4} of the initial rock weight. Moreover, high accuracy and precision are requested for such determinations, making even more difficult the development of proper instrument.

In this contribution, we present a new developed apparatus for the analysis of COH fluids in capsules deriving from rocks synthesis experiments, which allows the quantitative determination of volatiles in the fluid by means of a home-made capsule-piercing device connected to a quadrupole mass spectrometer. This strategy allows the analysis of all the gases delivered from rocks, and not only of a part of them, leading to the high sensibilies required to reach the targeted issues.

A quantitative analysis of COH fluids along with associated statistical errors is obtained by matrix linear regression of the m/z data of the sample and of standard gas mixtures of known composition. Confirmatory experiments on well-known systems were conducted to verify the absence of bias.

The estimated uncertainties fulfill the initial requests as they are typically <1% for H_2O and CO_2, and <5% for CO. Preliminary results one some interesting systems will be also presented.

Solid-phase Extraction of Trace Glucocorticoids from Environmental Waters on Silica-supported Humic Acids-derived Carbons Followed by HPLC-ESI-MS

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Being widely employed both in human and veterinary medicine, largely excreted with no structural modification, and not quantitatively abated by wastewater treatment plants, GCs are major emerging pollutants and their environmental widespread is reason of great concern due to their persistence and adverse effects, mainly endocrine disruption[1]. The typical concentrations levels detectable in environmental waters almost range from few to some hundred nanograms per litre[1,2], thus requiring a pre-concentration step before instrumental analysis. In this work, micrometric silica was modified with a carbonaceous phase obtained by thermally treated commercial HAs (600°C, N_2 flow, 1 h), previously physisorbed on the inorganic support. The obtained material (10 wt% HA@silica) was tested as a novel carbon-based sorbent for SPE of eight glucocorticoids (GCs) from environmental waters. Pre-concentration tests were undertaken on tap water samples (50 mL) enriched with 0.5-5 µg/L of each GC (prednisolone, prednisone, hydrocortisone, cortisone, betamethasone, dexamethasone, triamcinolone acetonide, fluocinoloneacetonide acetate), using 200 mg of sorbent packed in 3 mL SPE tubes (flow rate ~ 5 mL/min). Quantitative adsorption was observed at the sample native pH for all the analytes, which were simultaneously eluted in a single fraction (2.5 mL methanol) gaining quantitative recovery (90-110%, *n*=3). The HA@silica cartridge proved to be reusable for at least three pre-concentrations, with unchanged performance. The SPE procedure was also tested in raw river water samples, fortified with the target compounds at concentration levels in the range 50-500 ng/L, providing good recovery (70-112%, *n*=3) and enrichment factors (e.f.) up to 200. The lowest concentration detectable can be further improved with e.f. up to 2000 by evaporation of the eluate under N_2 stream. After SPE, GCs were separated and quantified by HPLC-ESI-MS/MS, obtaining good linearity in the range 10-100 µg/L, and specific detection (MRM mode). Further tests are ongoing on seawater and wastewater treatment plant effluents in order to investigate the applicability of the proposed SPE material to various/complex environmental matrices. A full characterization of HA@silica, including scanning electron microscopy, thermogravimetric analysis, Fourier transform infrared spectroscopy, BET surface area measurements, X-ray photoelectron spectroscopy, is now in progress. The main figures of merit, that is selectivity, sensitivity, linearity, recovery, precision and batch-to-batch reproducibility will be evaluated, and the final analytical method will be applied to the analysis of actual-world water samples. In view of the good performance of HA@silica, this material could be tested also for pre-concentration of other classes of emerging pollutants.

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Aliphatic Amines in Atmospheric Aerosol: Sources and Distribution

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Amines are atmospherically-relevant organic compounds, contributing to new particle formation[1] and to the production of toxic species[2]. They are mainly present in the gaseous phase, but they are partially converted into particulate phase after neutralization. The most common and abundant amines observed in particulate matter are aliphatic low-molecular-weight amines[2,3]. More than one hundred of different molecules belong to this class, the most abundant of which are Ethyl-amine (EA), Methyl-amine (MA), Diethyl-amine (DEA), Trimethyl-amine (TMA) and Dimethyl-amine (DMA). Till now different sources have been identified, which are mainly located in rural areas, such as animal husbandry, agricultural activities, biomass burning. Other minor sources are expected to be treatment of sewage and waste, combustion, industry, automobiles, cooking, tobacco smokes, composting operations and natural sources such as oceans, biodegradation of organic matter (that contains amino acids) and geologic sources[3]. The class of methyl amines is the most common in the atmosphere and has the highest concentrations near major sources, with a total global emission of 285 Gg N year^{-1}. TMA, the prevalent amine in the atmosphere, is estimated to have a global flux at approximately 170 Gg N year^{-1}, which is the highest among all alkylamines. It's believed that most of this flux comes from animal husbandry activities[3,4]. This study analyzes the spatial and seasonal variation of aliphatic amines in one of the european areas characterize by high nitrogen emissions[5]. Pm$_{10}$ samples were collected in northern Italy in one urban site in Milan and in two rural sites in the southern part of Lombardy (corte dei cortesi - cdc and schivenoglia), characterized by intense agricultural and zootechnical activities. Nitrogen organic species were first investigated by Fourier-transform infrared spectrometry. Then, aliphatic amines were quantified by ion chromatography (IC)[6]. After the set-up of the methodology, chromatography separation was achieved using a methanesulfonic acid (MSA) gradient elution on a dionex cs17 column with conductivity suppressor (dionex cprs 500), which has allowed the quantification of the main cations as well. The analytical results show higher concentration in the rural sites than at the urban one. Concentration of single amine compounds can be as high as 100 ng m^{-3}. The correlation of MA with K$^+$ and levoglucosan, tracers of biomass burning, suggests that one of the most important sources for this amine is residential heating. In the rural site, an increase in the DMA concentration has been observed (up to 36 ng m^{-3}) in early summer, likely due to the use of pesticides (indeed DMA is a component of pesticides). Amines can potentially be significant markers to track the impact of agricultural emissions and wood burning on air quality.

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Elemental and Organic Carbon contribution to atmospheric aerosol at Ny Ålesund (Svalbard Islands)

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Substantial climatic changes are affecting the Arctic since the past decades and, in this context, the atmospheric aerosol plays a key role in the current climatic changes, influencing the energy budget of the surface and lowermost atmosphere. In particular, during polar sunrise, the Arctic troposphere is a unique chemical reactor, due to emissions both from human activity in the mid/high latitude areas and from natural sources in the Arctic Ocean.

Moreover, light-induced chemical reactions are able to cause changes in atmospheric composition. The coupled effect of meteorological patterns and extreme variations in solar irradiance starting in spring makes difficult to unravel the factors of the production and removal of many environmentally relevant chemical compounds.

At this purpose, daily PM10 and size-segregated (by 4-stage impactor) aerosol samples were continuously collected at Gruvebadet station (Ny Ålesund, Svalbard Islands, 78.9°N, 11.9°E) during the spring-summer period (March-September) along six consecutive years (2010-2015) and were analysed for ion, metal and EC/OC (Elemental/Organic carbon) content.

Here we focus on the EC and OC fraction of the aerosol and on selected organic anions (oxalate, acetate, propionate, formate, glycolate and pyruvate), because of their importance in the absorption of solar radiation (EC) and their sensitivity to oxidation processes and to the dynamic atmospheric production/consumption triggered and/or mediated by solar radiation (OC and organic compounds). Most of the determined organic anions show very low concentration in all the years, with the exception of oxalate, which is always dominant among the organic anions.

The temporal trends of the measured compounds is studied in comparison both with the broad band UV solar irradiance data in order to evaluate the effect of the photochemistry on the studied compounds.

Moreover, correlations among different chemical markers are studied, together with backtrajectory analysis, in order to highlight the long-range transport events delivering these compounds together with pollutants from the anthropised areas of the Northern hemisphere. For instance, in particular periods, significant correlation between nitrate and organic acids are found, whose nature is still to be ascertained and which could be related to common photochemical production processes. Besides, the similarity in the spring pattern of sulphate and oxalate hints at a common anthropogenic source of the latter in this period and requires further investigation.

Co-localization of DNA-based conformational switches for Single-Step Fluorescence Detection of Antibodies

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Antibody detection is crucial for the diagnosis of many human disorders including infectious, autoimmune, and oncological diseases[1]. Moreover, since immunotherapy represents nowadays the frontier to fight certain diseases, the detection of specific antibody becomes of paramount importance to correctly monitor the progression and efficacy of such therapies. To allow early diagnosis, prompt therapeutic actions and efficient immune-based therapy monitoring antibodies detection methods should be sensitive, quantitative and specific but also rapid and easy to use[2]. Unfortunately, however, current methods routinely used for this purpose in clinical settings either require reagent-intensive laboratory-based techniques (ELISA and other heterogeneous, sandwich-type assays), multiple time-consuming incubation steps (e.g. Western Blot assay, radioimmunoassay), and/or sophisticated equipment (e.g. surface plasmon resonance).

In an attempt to find new solutions to this problem here we propose a novel approach for the rapid, sensitive and single-step detection of antibodies that couple the advantageous features of DNA-based conformational switching probes with those of co-localization based approaches. Specifically, we take advantage of the spatial geometry, that is shared by all IgG and IgE antibodies (i.e. the majority of diagnostically relevant antibodies) to co-localize on the same antibody DNA-based elements that are rationally designed to give a measurable fluorescence signal only in the presence of a specific target antibody. We also demonstrated the possibility of multiplex detection of antibodies in the same solution using different sets of DNA-based elements. In an attempt to find the best practical application for our platform we have also demonstrated the ability on the use of this strategy for the monitoring of the immune response of patients that are under vaccine treatment.

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Colloidal Nanoparticle modified Graphene-based electrochemical platforms for clinical biomarker detection

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Nowadays, graphene-modified electrodes and their application to electrochemical sensing have attracted enormous interest. The main advantages of graphene-based electrodes are a large potential window, fast heterogeneous electron transfer kinetics for various analytes and the involvement of additional "chemical features" (e.g. aromatic structures strongly connecting graphene via $\pi-\pi$ stacking interactions). The integration of Au nanoparticles (NPs) into graphene nanoflakes allows in certain cases to efficient improvement of the device sensitivity due to enhancement of the electron transfer or through its surface catalytic reaction with certain analytes (e.g. hydrogen peroxide). DNA sensing is another application of Au NP/graphene hybrids. The behavior of a novel AuNPs-graphene nanostructure was, herein, investigated towards electrochemical sensing of human metabolites[1] and towards the development of a DNA-based biosensor (genosensor). In particular, the novel electrochemical platform was evaluated for the development of a genosensor for the detection of miRNAs, a family of small, non-coding RNAs that are considered important clinical biomarker candidates for many diseases, including cancer[2]. Electrochemical techniques, such as Electrochemical Impedance Spectroscopy (EIS), Scanning Electrochemistry Microscopy (SECM) and Differential Pulse Voltammetry (DPV), were used for the development and the characterization of the biosensing platform. In a first attempt, the DNA capture probes were immobilized onto the electrode surfaces. Afterwards, the target biotinylated RNA was hybridized with the specific capture probes. The biosensing platform was then incubated with a streptavidin-enzyme-conjugate and exposed to a proper substrate. The product of the enzymatic reaction was electrochemically monitored by DPV. In a further approach, EIS was used to develop a label-free method.

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Smartphone–based enzymatic biosensor for rapid detection of oxidase substrates using confined multilayer paper reflectometry

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The development of smartphone–based biosensors for point-of-care testing (POCT) applications allows realizing "all in one" instruments, with large potential distribution among the general population[1]. The advance in complementary metal oxide semiconductor (CMOS) camera technology makes the smartphone suitable as a detector for reflectance measurements that is the most popular, simple, inexpensive and straightforward method to detect paper color-based assays[2]. Despite the increasing interest of recent years in the development of color change paper-based biosensors, they still suffer from a poor detectability and reproducibility related to inhomogeneity of color development, which leads to a poor quantitative analysis performance. This issue can be attributed to the mobility of enzymes and reagents towards the edge of detection zone when the sample is applied onto paper surface. To overcome these problems, we propose a smartphone paper-based biosensor, in which all the reagents necessary to complete the analysis are co-entrapped on paper in a "wafer"-like bilayer film of polyelectrolytes (Poly (allyl amine hydrochloride/poly (sodium 4-styrene sulfonate))[3]. The sequential adsorption of oppositely charged polyelectrolytes by layer-by-layer (LbL) deposition technique was exploited to obtain self-assembled polyelectrolyte multilayer thin films. The main advantages of this method are the possibility to control the structure and the total film thickness on a molecular level and the efficient selective entrapment of biomolecules such as enzymes exploiting non-covalent bonding and preserving biological activity in their microenvironment. The smartphone-based device was realized using a 3D printing low-cost technology. Its components are a semi-cover accessory attached to the smartphone, a polydimethylsiloxane (PDMS) light diffuser placed in front of the smartphone flash LED to improve the image quality, a mini dark box and a disposable analytical cartridge containing the bioactive paper-based support necessary for the analysis. The biosensor was developed exploiting coupled enzyme reactions for quantifying oxidase substrates such as L-lactate in oral fluid, which is considered a biomarker of poor tissue perfusion in sports performance evaluation. The developed method is sensitive, rapid and it allows detecting L-lactate in the relevant physiological range, with a limit of detection of 0.1 mmol L^{-1}. The extreme simplicity of assay execution (no reagents need to be added) together with the high versatility (the assay is HRP-based color change detection of H_2O_2 produced by an analyte-specific oxidase), make our approach generally applied for all oxidases, thus enabling the development of other specific bioassays to quantify several biomarkers such as glucose, cholesterol, ethanol, cortisol, xanthine and bilirubin.

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A Reagentless Paper-based Screen-printed Device to Detect Chloride Ions in Serum and Sweat

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Screen-printed electrodes (SPEs) are the ideal device to detect a plethora of species, but they often require additional tasks by the operator and their disposal is not always "green". We report a novel paper-based SPE for the detection of chloride in biological samples. With an easy manufacturing methodology, we developed a one shot and reagent-free electrochemical platform to selectively detect chloride ions in serum and sweat. To this purpose, a silver-based three-electrode configuration is utilized. The detection mechanism is based on the oxidation of the Ag contained in the ink to Ag^+, which reacts with the chloride (Cl^-) present in the sample. The formed silver chloride (AgCl) is successively reduced at the working electrode and these processes produce an oxidation and a reduction wave in the cyclic voltammogram curve, used to quantify the level of Cl^-. Chloride has been detected linearly up to 200 mM with a detection limit equal to 1 mM and a repeatability lower than 10%. The accuracy of the sensor has been evaluated in serum and sweat samples, achieving recovery values ranging from 93 ± 10 % and 108 ± 8 %. Moreover, the analysis of five serum samples provided by the Hospital gave comparable results with respect to the gold standard method (Ion Selective Electrode) adopted in routine clinical analyses, demonstrating the suitability of this paper-based device.

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PVA/GO/Au: Development and Characterization of a New Electrode Material

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In the last years the use of nanostructuring materials has increasingly spread in many scientific and applied technologies fields. Chemically modified electrodes (CMEs) can be made using nanomaterials, organic monolayers or composite hybrid system developed by fusion of organic polymers and nanostructured electroactive species. These different strategies for modifying electrodes provide opportunities to confer a unique range of properties to electrode surfaces, like an improvement of electrode surface areas or electrocatalytic behavior, obtaining electrodes with useful switchable properties[1]. Nanomaterials are often made by carbon nanostructures or metallic nanoparticles not only as pure metal but also as alloys and/or oxihydroxide forms. Carbon nanostructures, like graphene, graphene oxide, and nanotubes, are widely used because of their interesting properties such as large specific surface area, high thermal and electrical conductivities, great mechanical strength and low cost[2,3]. Many transition metals show interesting electrochemical properties in terms of conductibility, stability and catalytic activities. In this respect, Au species present important electrocatalytic performance toward the electrooxidation of various organic molecules in aqueous medium[4,5]. In this study, we develop a new CME using a composite active material based on graphene oxide dispersed within the polyvinyl alcohol (PVA) and doped with electrodeposited Au particles (PVA/GO/Au). The PVA, although is scarcely employed in electrochemical applications, represents a versatile and interesting polymer employed in many fields of applied technology, such as medicine[6], and anti-corrosion coating polymer[7].

The glassy carbon was modified by casting 10 μL GO/PVA emulsion on the surface electrode and successively, Au particles were electrodeposited using an optimized pulsed electrochemical procedure by buffer carbonate solutions (pH= 8) containing 5.5 mM $HAuCl_4$.

The PVA/GO/Au electrode was electrochemically characterized as electrocatalyst toward the electrooxidation of alditols and carbohydrates in alkaline medium. Scanning electron microscopy (SEM) and X-Ray photoelectron spectroscopy (XPS) were used in order to characterize the morphology and the chemical speciation of the electrode surface. In addition, the modified electrode was characterized as a capacitor system during continuous charge-discharge process in alkaline medium.

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Glucose/Oxygen Enzymatic Fuel Cell Based on a "Green" Gold Nanoparticles Modified Graphene Screen-Printed Electrode for Glucose Detection in Human Saliva

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The aim of this work is to realize a miniaturized high-power glucose/oxygen enzymatic fuel cell (EFC) based on efficient DETs, employing cellobiose dehydrogenase (CDH) and laccase, respectively, and using a single screen-printed graphene electrode modified with "green" AuNPs. In fact, among the enzymes able to perform DET with the electrode surface, CDH is receiving great attention thanks to its ability to show DET upon reaction with many substrates including sugars, such as glucose and lactose[1,2].
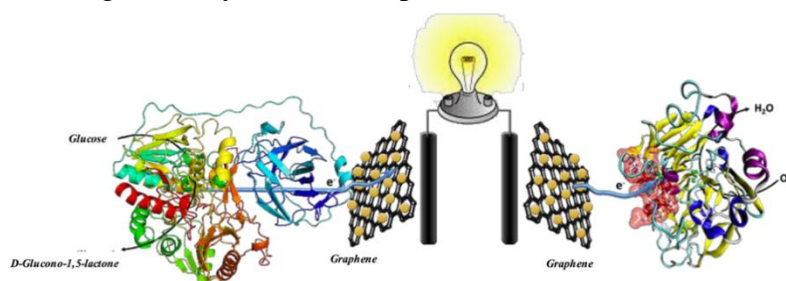
The bioanode and biocathode are a graphene working electrode and a graphite counter electrode localized on the same screen printed electrode (SPE) modified with poly (vinyl alcohol) N-methyl-4(4'-formylstyryl) pyridinium methosulfate acetal (PVA-SbQ)/cellobiose dehydrogenase from *Corynascus thermophilus* (CtCDH) C291Y/AuNPs and with *Trametes hirsuta* laccase (ThLac)/AuNPs, respectively. In order to optimize the bioanode, several CDH immobilization procedures were adopted, such as drop-casting, use of Nafion membrane or PVA-SbQ photopolymer. The photopolymer showed the best performances due to its high internal flexibility allowing an easy re-orientation of the enzyme through the electrostatic interactions between enzyme and gold nanoparticles (AuNPs)[3]. Furthermore, the use of the nanomaterials allowed to reduce the overpotential of O_2/H_2O redox reaction catalyzed by *Trametes hirsuta* Laccase (ThLac), drop-casted onto the AuNPs modified SPE. The performances of bioanode and biocathode were tested separately, initially immobilizing both enzymes onto separated graphene SPEs. Successively, the bioanode and biocathode were assembled in a non-compartmentalized EFC and a remarkable 50% decrease of the maximum power output at the value of 2.15 ± 0.12 µW cm^{-2} at cell voltage of 0.48 V and an OCV of 0.62 V at pH 6.5 was registered. In order to miniaturize the device, the bioanode and biocaythode were realized by immobilization of both enzymes onto the same SPE. The so miniaturized EFC delivered maximal power output of 1.57 ± 0.07 µW cm^{-2} and 1.10 ± 0.12 µW cm^{-2} with an OCV of 0.58 V and 0.41 V in a 100 µM glucose solution and in human saliva, respectively[4].

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Generation IV Ionic Liquids and Nanomaterials to Develop Innovative Electrochemical Biosensing Platforms

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Different types of modified electrodes have been prepared and tested to assembly and construct portable sensing devices, useful to analyze, in a simple, fast and economic way, many analytes.

Bare glassy carbon electrodes (GCE) of the screen-printed type (SPE), with silver electrode as reference, have been modified, via drop casting on the working electrode surface, with a mixture of generation IV room temperature ionic liquids (RTILs) and nanomaterials.

The ionic liquids choline-amino acids, were synthetized in our laboratory following the methodology known in literature[1]. These RTILs differ from the previous generation ones because they are nontoxic and completely biodegradable[2,3]. Like those of the previous generations, the RTILs used in this work have interesting properties regarding low volatility, low flammability, high ionic conductivity, high chemical and electrochemical stability[4]. Furthermore, these compounds allow to significantly increase the work potential range (V), electroactive area (A_{ea}) and electron transfer constant (k_0), so that anode or cathode peaks can be detected even for extremely low concentration of analytes.

The nanomaterials used in this work were Multi-Walled Carbon Nanotubes (MWCNT) and Titanium Dioxide nanoparticles (anatase form). These two nanomaterials have been used both alone and together in different proportions with the chosen RTIL [Ch] [Phe]. All the so developed electrodes have been studied by means of cyclic voltammetry measurements to calculate the electroactive area (A_{ea}) of the working electrode, in order to find the best combination of nanomaterials/ionic liquid/water for the drop casting mixture.

All the proposed combinations have also been studied for stability: cyclic voltammetry measurements have been carried out at various time intervals after the preparation to investigate variations of the A_{ea} of the modified electrodes.

The electrochemical platform so developed has been used, after functionalization with the proper enzyme, as a biosensor. In particular, it has been tested for measuring glucose in food matrices and antioxidants in olive oils using Glucose Oxidase[5] and Lipase[6] respectively.

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Identification of bioactive peptides derived from cauliflower by-products by multidimensional liquid chromatography and bioinformatics

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Brassica crops, a huge vegetable family mainly constituted by cauliflower and broccoli, have a world production of about 22.3 million tons. Most of the production comes from Asia (with 82% of the total production), whereas Italy is the second world producer (with 0.4 million tons), followed by Spain and Mexico (http://faostat3.fao.org/). As a consequence of this intensive production, tons of cauliflower by-products are generated every year, and they are mainly made up of leaves (50% of the total) and stems. These residues might cause huge economic and environmental problems, and that is the reason why it is important to discover new uses of these waste materials, outside the agricultural sector as well, in order to create sustainable value chains in the farming and processing sectors[1].

At the same time the use of food industry-related by-products is also gaining interest, since it is now well known that some specific residues generated during food manufacture possess significant amounts of bioactive compounds. Correlated to Brassica crop, recent works have shown that cauliflower by-products are a good source of phenolic compounds, mainly flavonol derivatives[2]. While many studies reported the presence of AO and anticancer compounds in broccoli[3], the bioactive peptides (BAPs) remain poorly investigated.

The main objective of this study was the development of a new systematic analytical approach for the recovery, separation, identification of BAPs. In particular, in this study, we employed a platform based on two different extraction methods, comparing a sodium dodecyl sulphate (SDS) and ethylenediaminetetraacetic acid (EDTA) based protocols, followed by an in vitro digestion by two different enzymes, trypsin and a mixture of pancreatin and pepsin. The four protocols were tested for the ACE-inhibitory and AO activity. The protocols providing the highest bioactivity were purified by a combination of consecutive chromatographic separations, first by RP liquid chromatography and then by hydrophilic interaction chromatography (HILIC). The most active fractions, obtained from the second chromatographic dimension, were further analysed by nanoRP-HPLC coupled with an Orbitrap mass spectrometry for peptide sequencing. After database search, the identified peptides were further mined by in-silico analysis using PeptideRanker, which provided a bioactivity score later used to select candidates for chemical synthesis. Finally, on the basis on the probability as calculated by PeptideRanker algorithm and on the area, 6 peptides were selected and synthesized. The synthesized peptides were validated compared to the natural occurring ones checking their retention times and fragmentation patterns in cauliflower by-product hydrolysates. Finally, their activities were determined by in-vitro bioassays on the individual peptide standards.

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Solid phase extraction of dibenzyl disulphide on a molecularly imprinted polymer

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Dibenzyl disulphide (DBDS) is commonly present in power transformer oils as an antioxidant at concentration of around 100 ppm. Only recently it has been found to produce damages of these devices since it is corrosive for copper and cellulosic components[1]. It is possible, even if not easy, to control the composition of the oil in a transformer, so that the determination of DBDS in this particular matrix is of great interest for the prevention of the transformers failures. Many analytical methods have been proposed, based on chromatography with previous separation by liquid and solid phase extraction (SPE) with different sorbents[2]. Here a molecular imprinted polymer (MIP) is proposed for the first time for the selective extraction of DBDS from a power transformer oil. The procedure has been characterized by a new electrochemical method for the determination of DBDS in acetonitrile-ethanol (80:20) solution (DPV method), which has been purposely developed. This particular sorbent was considered because of its high selectivity which would allow an electrochemical determination without further separation. A scheme of the analytical procedure is reported below:
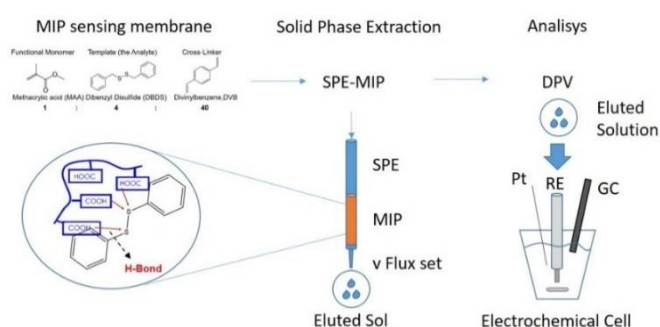
For the preliminary investigation, MIP was synthesized according to a previously described procedure[3], and then mechanically pulverized to particles of about 50 μm. DBDS was extracted from the polymer by repeated washing with ethanol and hexane. Successive extractions were controlled by UV, DPV, and EDS. MIP capacity in acetonitrile-ethanol (80:20) solvent at concentration of about 100 ppm is $2.4 \cdot 10^{-3}$ mmol/gMIP, with a distribution coefficient K_D equal to 7. The adsorption of DBDS by column procedure was investigated by determining the breakthrough curve of a solution with 32 ppm of DBDS, on a column constituted by 0.2 g of pulverized MIP, and at elution rate of 0.2 ml/min[4]. The DBDS separation from a widely used transformer oil (Nytro Libra insulating oil from Nynas S.r.L. (Stockholm, Sweden) was successfully performed on the column described at the following conditions: 0.5 ml of oil introduced, 2 ml of acetonitrile-ethanol (80:20) solution for washing, 5 ml of acetonitrile-ethanol solution (80:20) for elution (110% recovery).

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Analytical Characterization of New Composites for Food Packaging Applications

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The application of metals and metal oxides in the form of nanostructured antimicrobials is continuously expanding. In the last decades, especially Cu- and Ag-based nanoantimicrobials [1] have attracted the interest of scientists and technologists, as assessed by the huge amount of reports on their synthesis and characterization [2]. Designing bioactive materials, with controlled metal ion release, exerting significant biological action and associated low toxicity for humans, is nowadays one of the most important challenges for our community. The most looked-for nanoantimicrobials are capable of releasing metal species with defined kinetic profiles, either slowing down or inhibiting bacterial growth and pathogenic microorganism diffusion [3]. In recent years, we have developed and deeply characterized many different nanoantimicrobial systems, ranging from Ag- and Cu-modified textiles [3] to bioactive food packaging [4]. On the one hand, all these materials were found to be human safe, since they showed no significant leaching of (potentially toxic) whole nanoparticles into contact media. On the other hand, antibacterial ionic species released by transition metal nanophases provided a powerful alternative route to fight bacterial resistance towards conventional antibiotics and disinfecting agents. Laser ablation synthesis in solution (LASiS), in particular, has been used in our labs to produce bioactive Cu- and Ag-based nanocolloids, both in aqueous and organic media, which can be used as water-insoluble nano-reservoirs in composite materials [5]. In fact, including nanophases into polymers (like polyethylene oxide or polylactic acid) allows to produce multifunctional packaging, combining biodegradable and antibacterial properties of both organic and inorganic phases. In this study, x-ray photoelectron spectroscopy was used to evaluate the chemical speciation and elemental composition of pristine nanoparticles and final materials, providing useful information about synthesis processes, as well as storage and processing conditions. Transmission electron microscopy was exploited to study morphologies of nano-colloids, along with UV-Vis and infrared spectroscopies for bulk chemical characterization. Electrothermal atomic absorption spectroscopy was used to investigate metal ion release from modified surfaces and industrial products. Analytical spectroscopy results were matched with bioactivity tests on target microorganisms of food spoilage. Different approaches to the synthesis and characterization of second-generation nanoantimicrobials, e.g. to those materials combining antibacterial efficiency and nanosafety issues, will be critically discussed, based on spectroscopic, morphological, release and bioactivity evidences.

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Photocatalytic Transformation of C_2Cl_4 in Gas Phase Under UV-irradiated TiO_2

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The photocatalytic transformation of chlorinated solvents has been the topic of numerous works since the first studies of the photocatalytic processes under irradiated semiconductors. As an example, in the photocatalyzed transformation of perchloroethylene (PCE) in the presence of oxygen the $Cl^{\bullet}$ radical has supposed to have a pivotal role in the first step of the degradation and in the successive transformation of the PCE by-products[1]. Also in aqueous solution, the role of halogen radicals was advanced to explain the formation of CCl_4 from chlorinated methanes[2].

In this work, we investigated the photodegradation of gaseous PCE on titanium dioxide films under UV light at 365 nm at the gas/solid interface. PCE conversion was carried out in a CSTR photo-reactor (Continuous Stirred Tank Reactor)[3] and the residual PCE leaving the reactor was properly detected using a PID detector and FTIR. The C_2Cl_4 photocatalytic transformation was investigated at different experimental conditions, such as at different PCE concentration, TiO_2 irradiated area, inlet gas flow and UV irradiance ($W \cdot m^{-2}$). The water vapor has a detrimental role on the PCE transformation rate due to the competition with PCE adsorption on reactive sites.

The rate and mechanism of transformation change when oxygen is present at high and negligible concentrations. Gaseous products of PCE degradation were determined: CO_2, phosgene, carbon tetrachloride (CCl_4), hexachloroethane (C_2Cl_6) and, at lower concentrations, trichloroacetyl chloride (TCAC) were identified as photodegradation products. In quasi-anoxic conditions, significant amount of adsorbed organic compounds with molecular weights higher than that of PCE were produced on the UV-irradiated TiO_2 surface and chloride ions are more accumulated at the surface, proof of the presence of a predominant reductive pathway. However, the addition of chloride ions on TiO_2 surface does not increase the production of gaseous CCl_4.

The production of active chlorine (sum of Cl_2, $HClO$ and $ClO^{\bullet}$) was investigated both in the presence of oxygen and in quasi-anoxic conditions. At low O_2 concentration no gaseous active chlorine was determined, while a significant amount was measured in the presence of oxygen. By considering that in the absence of O_2 the only possible form of active chlorine is Cl_2, the experimental evidences underline that the produced active chlorine is $HClO/ClO^{\bullet}$. Then chlorine radical has a minor role in the abatement of C_2Cl_4.

These experimental data allowed us the identification of the main photodegradation pathways of PCE at high and low oxygen concentration giving intriguing and new insights into the photocatalytic transformation of chlorinated olefins from gaseous phase, putting the role of chloride radicals as reactive species back in a different perspective.

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Microscopic and Spectroscopic Analysis with Synchrotron Light of Modified Surfaces of Technological Interest

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A fundamental aim of material sciences is to reckon the relationship between the properties of a device, and the morphological and structural characteristics of the surface. Combining basic electrochemical techniques with spectroscopic, microscopic and structural techniques is crucial for characterizing the structure-activity relationship for many different technological devices. We consider this approach even more interesting, if these electrochemical and structural characterizations are performed simultaneously under the control of the electrical potential. In this context during the last two decades, several interesting combined techniques emerged. In this talk we will present in-situ studies of electrochemical-spectroscopic, electrochemical-microscopic and electrochemical techniques combined with synchrotron radiation. In particular, technical EC-SPM (Electrochemical- Scanning Probe Microscopy) and EC-SRM (Electrochemical-Synchrotron Radiation methodologies) will be presented. We will present operando studies carried out employing these techniques to show the variation of the chemical, morphological and structural aspects of surfaces during straightforward electrochemical processes or by simply unplugging the electrical potential. The industrial impact, elicited by this experimental approach, will be also presented.

Metodi multi-block per l'analisi degli alimenti

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Analizzando dati chimici, qualora si abbia a disposizione un data-set con diversi blocchi di dati (usati come predittori in un modello di regressione o di classificazione), è stato dimostrato che sia più conveniente utilizzare degli approcci *multi-block* (detti anche di *data-fusion)*, ovvero dei metodi che permettano l'estrazione delle informazioni dai diversi blocchi di dati contemporaneamente, piuttosto che analizzarli individualmente (1). In chimica degli alimenti, accade sovente di avere dei data-set multi-block, principalmente perché gli stessi campioni possono essere analizzati con diverse tecniche analitiche. Numerose applicazioni di metodologie di *data-fusion* sono state effettuate in ambito alimentare; nel presente lavoro, particolare interesse verrà rivolto verso l'autenticazione e la classificazione di una birra artigianale prodotta nel Lazio (Birra del Borgo, Borgorose (RI)). I campioni sono stati analizzati mediante cinque differenti tecniche analitiche: Spettroscopia MIR, Spettroscopia NIR, Spettroscopia UV, Spettroscopia Visibile e termogravimetria. Le matrici di dati sono state utilizzate sia individualmente che congiuntamente al fine di realizzare i differenti modelli di classificazione (2). Per la creazione dei modelli con i blocchi individuali si è utilizzato PLS-DA (3), mentre per l'analisi multi-block dei dati sono stati utilizzati gli approcci Low- e Mid-Level data fusion (4) e Sequential and Orthogonalized-PLS (SO-PLS) combinati con la Linear Discriminant Analysis (LDA) (6-8). I risultati così ottenuti sono stati studiati sia dal punto di vista predittivo che dal punto di vista dell'interpretazione. In generale, tutti i metodi multi-block hanno fornito predizioni soddisfacenti, migliori dei risultati ottenuti mediante classificazione sui blocchi individuali; in particolare, il Mid-Level data fusion ha permesso di ottenere il 100% di corretta classificazione per tutti i campioni di test (una rappresentazione grafica dei risultati ottenuti mediante questo metodo classificativo è riportato in Figura 1). Dal punto di vista interpretativo, i modelli basati sul metodo SO-PLS hanno mostrato degli evidenti vantaggi.
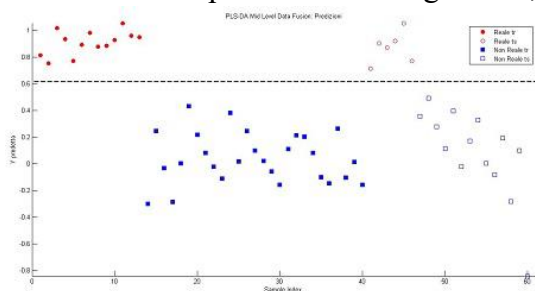
Figura 1: Predizioni Modello PLS-DA Mid Level Data Fusion

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An Innovative NIR Hyperspectral Imaging Approach to Pattern Cheese Ripening

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Biochemistry of cheese ripening is considered under two general headings: proteolysis and lipolysis. Proteolysis is the most complex biochemical activity that contributes to cheese ripening and flavour development through the formation of peptides and AA and by changing the texture of cheese from the breakdown of the protein network. Lipolysis in cheese is due to the presence of lipolytic enzymes that cleave the ester linkage between a fatty acid and the glycerol moiety of a triacylglycerol; it produces free fatty acids (FFA) with chain lengths $\geq$C4, glycerol, and mono- and diacylglycerols.

Several analytical methods are available to evaluate cheese ripening ranging from qualitative approaches, aimed at following process evolution, to quantitative approaches, which allow to quantify compounds being formed. In this work two reference methods were applied on several sub-samples radially located within cheese specimens to evaluate the degree of protein hydrolysis (DH) and the total free fatty acid (FFA) content – o-phthaldialdehyde (OPA) spectrophotometric assay and copper soap method, respectively. However, no technique was proposed yet to pattern cheese ripening, which is clearly influenced by location within a cheese and tends to evolve following a radial pathway from the centre to the borders of the cheese. Until now, a suitable sampling scheme has been the only prospective to account for such differences.

The aim of this work was the reliability evaluation of near infrared hyperspectral imaging (1000–2500 nm), to monitor not only the evolution but also the spatial extent of proteolysis and lipolysis in cheese. First of all, a qualitative assessment of the NIR bands associated with peptides and free fatty acids was carried out on a wide set of cheese, characterized by different ripening types: mainly proteolytic or mainly lipolytic. Then, a semisolid slicing cheese was followed during the early stages of its ripening, acquiring hyperspectral images daily for a total of 10 days from cheese production (three samples each day).

After the deploying of suitable pre-processing methods for minimising the amount of useless information within hyperspectral data, multivariate pattern recognition, both exploratory and supervised, allowed to efficiently map ripening across sample surfaces. In particular, principal component analysis (PCA), integrated with a brushing approach between score images and score plots, allowed to efficiently monitor cheese evolution and to characterise the different stages according to definite NIR absorption bands.

The results indicate that the NIR-HSI approach adds spatial information in cheese biochemical knowledges allowing to deeply understand the phenomena that occurs during cheese ripening.

Development of a method for the quality control of polystyrene (EPS) based on the use of microscopy techniques and multivariate statistical methods

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Synthered Expanded Polystyrene (EPS) is a widely-used polymer both for packaging and for the production of handworks, as containers, surfboards etc. According to the final application, the handwork itself must exhibit mechanical properties that make it suitable for the final purpose. The industrial quality control of EPS products for different applications is usually carried out by mechanical methods and technological measurements.

In this work, an alternative method has been developed for the industrial quality control of EPS products, based on the surface characterization of the object by microscopy coupled to multivariate statistics. The method has been applied to samples either classified by classical quality control methods as suitable (OK) or not suitable (KO) for the final application purpose.

In particular, the samples belonged to different production lots of surfboards and containers; for each type of product, OK and KO samples were available.

The surface of the samples was characterized by recording images through optical microscopy with different acquisition settings. The Zernike's mathematical moments were then calculated for each image to obtain global descriptors of the image features. The set of mathematical indexes thus calculated was therefore used to build multivariate classification models for a future application in industrial quality control. To this purpose different classification methods were compared (PLS-DA, SIMCA, LDA etc), also coupled to variable selection strategies. The applied method proved to be a valid alternative to classical quality control, allowing at the same time an easy automation of the overall procedure.

Application of an electro-activated glassy carbon electrode (GCE) to the analytical monitoring and photochemical studying of acetaminophen

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The occurrence of emerging contaminants in surface waters, such as pharmaceuticals and personal-care products, is an environmental issue of high concern due to the toxic effects on ecosystems and humans[1]. *Waste Water Treatment Plants* (WWTPs) are usually not able to remove these compounds from wastewater because they are water soluble and biorefractory. Therefore, emerging contaminants are often released in water bodies. As far as pharmaceuticals are concerned, antibiotics, analgesics and anticonvulsionants are usually detected in surface waters[2]. In these environments, xenobiotics can undergo both abiotic and biotic degradation. Among abiotic pathways, direct and indirect photochemical reactions can be key degradation processes. Direct photolysis occurs when the xenobiotic absorbs the solar light and undergoes subsequent chemical transformations. In contrast, indirect photolysis is triggered by *Photochemically Produced Reactive Intermediates* (PPRIs) that react with xenobiotics. The main PPRIs are $HO^\bullet$, $CO_3^{\bullet-}$, excited triplet states of Chromophoric Dissolved Organic Matter ($^3CDOM^*$) and $^1O_2$. They are formed upon absorption of solar light by photosensitizing compounds, such as CDOM, nitrate and nitrite[3]. Usually, photochemical reactions act as self-depuration processes in water bodies, but they can also induce formation of harmful photodegradation products. Luckily, the photochemical fate of xenobiotics (half-life times and photoproduction of intermediates) can be modeled, and thus foreseen, by knowing or measuring the relevant photoreactivity parameters (e.g., reactions rate constants and photoproducts formation yields) and by using suitable photochemical models[4]. Therefore, the monitoring of xenobiotics concentration in both WWTPs influents/effluents and surface waters, and the understanding of the photochemical fate of these compounds, are important tools to assess the human impact on aquatic environments. Acetaminophen (also known as paracetamol, hereafter APAP) is a widely used analgesic drug usually detected in surface waters[2] at concentrations up to $10 \ \mu g \ L^{-1}$ [5]. In this work, we have focused on the use of *Differential Pulse Voltammetry* (DPV), with a recently implemented electrochemically activated glassy carbon electrode (aGCE)[6], for the monitoring of APAP in the surface waters of the Po river basin, in the province of Turin (Piedmont, Italy). In order to confirm the reliability of the electrochemical response, we have compared DPV results with those obtained with *High Performance Liquid Chromatography* hyphenated with *High Resolution Mass Spectrometry* (HPLC-HRMS), commonly used to quantify pharmaceuticals. Furthermore, DPV with the aGCE was used to assess the photochemical degradation of APAP, and the relevant kinetic results were compared to those obtained in a previous work with a standard experimental methodology[7]. The purpose is to make it possible to monitor the APAP photodegradation, and the formation of photoproducts, in real-time by developing an adequate electrochemical set-up.

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Removal and photodegradation of glyphosate herbicide from waters using a mesoporous substrate with adsorbent properties

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Nowadays, a large amount of pesticides is used worldwide and many of these substances penetrate the soils, polluting water resources. Among these compounds, glyphosate is a non-selective herbicide recently classified as a probable carcinogen for humans (Group 2A). Conventional water treatment techniques are not effective in its removal, therefore the scientific community is exploring new approaches for the removal of glyphosate from aquatic systems.

In this work, we evaluate the adsorption and degradation properties towards glyphosate of a mesoporous silica-based support, encapsulated with iron oxides and functionalized with (3-aminopropyl)triethoxysilane (SBA-15-FexOy-NH$_2$). Results demonstrate the excellent adsorption performance of the SBA-15-FexOy-NH$_2$ towards glyphosate. Indeed, the adsorption is very fast and reaches equilibrium during the first 10 minutes of contact. Through the adsorption isotherms, the maximum monolayer coverage capacity (98 mg/g) was obtained. The adsorption properties of SBA-15-FexOy-NH$_2$ were also studied in the presence of high concentration of PO_4^{3-} (40 mg/L), a potential competing ion frequently present in water resources. Results indicate that the material keeps unaltered its adsorption performance, while at the same time removing PO_4^{3-} from the aqueous solution.

The presence of Fe in the substrate was intended to trigger photodegradation reactions; in particular, photo-Fenton reactions were studied at pH 3.0 and 6.5 under UVA and UVB irradiation. The most effective method was UVB irradiation at pH 6.5; these conditions allowed the successfully degradation (90%) of the previously adsorbed glyphosate. Worth noting it was observed the retention of the glyphosate by-products on the SBA-15-FexOy-NH$_2$ sorbent, thereby preventing the release of additional molecules into the aqueous matrix. It was shown that the photo-Fenton treatment regenerates the material and makes it possible to carry out at least three adsorption-degradation consecutive cycles, without any loss in performance.

Finally, the adsorption and photodegradation capabilities of the material have also been verified on a Po river water sample, thus showing the possible application of the sorbent even in matrices that are affected by high amounts of possible interfering species (e.g. organic matter).

The study here presented is part of a PhD research program.

Characterization of Ionic Composition in the Submicron Fraction of Urban Aerosol

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Atmospheric particles are polydisperse, varying the diameter of more than four orders of magnitude: from some nanometers (nm) to tens or hundreds of micrometers (μm)[1]. Particle size is one of the most important parameters to monitor particles' behavior in the atmosphere because it indicates the type of source and influences transport and deposition processes. Particle size distribution and concentrations of ionic species are quantified to determine their potential sources of input, chemical evolution and transport (2).

The main aim of the present work was to investigate the particle size distribution of ionic compounds (Cl^-, Br^-, I^-, NO_3^-, MSA, SO_4^{2-}, PO_4^{3-}, C_2-oxalic acid, C_2-glycolic acid, C_2-acetic acid, C_3-malonic acid, cis-usC_4-maleic, trans-usC_4-fumaric, C_4-succinic acid, αC_7-benzoic acid, C_5-glutaric acid, hC_4-malic acid, C_6-adipic acid, C_7-pimelic acid, Na^+, NH_4^+, K^+ and Mg^+) in urban aerosol collected at Venice. We used 120 MOUDI^{TM} II sampler that permitted to separate particles in 12 dimensional fractions starting with a diameter greater or equal to 18 μm to a diameter below of 56 nm. The sampling was carried out during the 2016 spring season between March and May.

The determination of anionic compounds was performed using ion-chromatography coupled to mass spectrometry (IC-MS), while cationic species were determined through capillary ion chromatography with conductometry[2].

The concentration of ionic compounds in the aerosol samples represented the highest percentage of aerosol mass concentration. The study of the ionic composition and their particle size distribution in the analyzed samples has allowed to identify different sources, as well as marine input and anthropogenic contribution.

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Sea Salt Sodium Record from Talos Dome (East Antarctica) as a Potential Proxy of the Antarctic Past Sea Ice Extent

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Sea-ice represents a powerful phenomenon exerting a strong influence on the oceanic, biological and climatic systems and, given its importance, it is a focus in environmental research. The expansion and retreat of Antarctic sea-ice is one of the most striking seasonal changes affecting the Earth today, effectively increasing by two-fold the surface area of Antarctica. Despite an unknown exact mechanism, sea-ice plays a critical role in the polar amplification of climate change and, therefore, it is important to predict changes in sea ice under the future conditions of an ongoing climate change. A better understanding of the links between sea-ice and climate and a real improvement of the models requires a large series of long observational datasets. Antarctic sea ice has shown an increasing trend in recent decades, but with strong regional differences from one sector to another of the Southern Ocean. The Ross Sea and the Indian sectors have seen an increase in sea ice during the satellite era (1979 onwards). Here we present a record of $ssNa^+$ flux in the Talos Dome region during a 25-year period spanning from 1979 to 2003, showing that this marker could be used as a potential proxy for reconstructing the sea ice extent in the Ross Sea and Western Pacific Ocean at least for recent decades. After finding a positive relationship between the maxima in sea ice extent for a 25-year period, we used this relationship in the TALDICE record in order to reconstruct the sea ice conditions over the 20th century. Our tentative reconstruction highlighted a decline in the sea ice extent (SIE) starting in the 1950s and pointed out a higher variability of SIE starting from the 1960s and that the largest sea ice extents of the last century occurred during the 1990s. Our findings were also compared to other ice core reconstructions of Antarctic past sea-ice extent.

A Six-year Record of Size Distribution and Chemical Composition of Arctic Aerosol. Main Results and Future Bi-polar Perspetives.

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The Arctic regions are among the areas most sensitive to present climate changes; through connections involving ocean, atmosphere, biosphere, lithosphere and cryosphere, they respond to, amplify, and drive changes elsewhere in the Earth system, so that understanding their role the climate-environmental feedback processes is essential in order to set reliable predictive climate models. In particular, the atmospheric aerosol strongly interacts with the climate forcings through scattering and absorption of the solar irradiation and as sources of cloud condensation nuclei. Although these processes are well known, the quantitative and qualitative (the sign of the climate forcing) of the aerosols in the Polar Regions is affected by a large uncertainty, yet. The main uncertainties include the relative cloud/snow surface albedo and the scarce spatial coverage of size distribution and chemical composition of aerosol at high latitudes. In order to improve our knowledge on the size distribution, the atmospheric load and the chemical composition of the Arctic aerosol, continuous measurements and sampling campaigns are ongoing since 2010 in two Arctic sites: Thule (North Greenland) and Ny Alesund (Svalbard Island, Norway). At Thule, 24-h samples are all-year-round collected at daily or every other day resolution. Contemporaneously, "summer" (March to September) aerosol was annually sampled at Ny Alesund. In the latter site, aerosol was collected by several systems (PM10 samplers, multi-stage impactors (4- and 12-stage Dekati samplers) and on different substrates (Teflon filters, quartz filters, polycarbonate and Teflon membranes). Besides, shorter measurement and sampling campaigns were carried out by using a tethered balloon, up to about 1.000 m altitude, in order to study the effect of the PBL dynamics on the aerosol atmospheric load and chemical composition. Filter chemical analysis includes: ions composition (inorganic anions and cations and selected organic anions, including light carboxylic acids and MSA), elemental composition (by PIXE analysis), main and trace metals (including Rare Earth Elements - REEs, by ICP-HR-MS), Pb isotopic ratios (by ICP-QMS) and Elemental/Organic Carbon fractions (EC/OC, by Sunset thermo-optical analysis). Besides, continuous measurements of particle size-distribution (TSI-SMPS and TSI-APS; 6 nm – 20um; 10 min resolution), Black Carbon (by Particle Soot Absorption Photometry – PSAP) and natural radioactivity (Rn progeny, by FAI PBL Monitor) were carried out during the sampling periods at Ny Alesund. Here we report the most relevant results up to now obtained and the future perspective both concerning Arctic and Antarctic aerosol, in the framework of three PNRA (Progetto Nazionale di Ricerche in Antartide) projects recently approved and funded.

In particular, the use of specific chemical markers (such as nss-sulfate for anthropic Arctic Haze, REEs elements for dust, MSA for biogenic emissions, selected heavy metals for local and long-range anthropic sources) allowed studying the changes in sources intensity and atmospheric transport efficiency for aerosol reaching the Arctic regions. Sulfate source apportionment was performed in order to evaluate the contribution of sea spray, continental dust, biogenic emissions and anthropic activities to the sulfate global budget. MSA was used in order to understand the complex interactions among sea ice dynamics, chlorophyll blooms and marine primary productivity. Lead isotopic composition and REEs composition were used in identifying the continental source of dust and anthropic pollutants. Finally, a relationship between marine biogenic emissions and nucleation events was found by particle size distribution in the nanometric mode.

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Effects of Biomass, Temperature and Thermochemical Process on PAH Concentration in Biochar

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Biochar is the solid carbonaceous material produced by the pyrolysis of biomass for a variety of applications in the agro/environmental field[1]. The thermochemical processes of biomass also produce a variety of organic compounds including PAHs, highly harmful compounds to man and to the environment.

The effects of biomass, temperature and type of process (pyrolysis and gasification) on 16 US EPA PAH concentration in biochar were investigated. To this end, 47 biochars were analyzed for their PAH content. 38 of them were produced in laboratory furnace, in highly controlled pyrolysis conditions, from 11 biomasses of which 7 were of wood (beech, chestnut, pine, poplar, spruce pine, commercial conifer, and pruning from fruit tree and grape vine) and 4 of grass (thistle, giant cane, rice straw and miscanthus leaves). The temperatures of pyrolysis were 350, 550, 750 and 1000 °C. In addition, 6 biochars were produced in industrial plants of pyro-gasification or pyrolysis. These plants were fueled with six of the biomasses used in the laboratory pyrolysis (spruce pine, conifer, pruning, thistle, giant cane and rice straw). Finally, 2 biochars (named STG and SPG), produced in a small experimental plant of gasification, and a biochar commercially available were considered. For the latter the production conditions were not known.

Examining the sum of the concentrations of 16 PAHs (Σ_{16}PAH) of each laboratory biochar, it can state that the type of biomass (wood or grass) does not affect the Σ_{16}PAH, but this Σ_{16}PAH depends on single biomass. Although the highest Σ_{16}PAH values related to the type of biomass were found at 350 °C for wood biochars and 550 °C for grass biochars. The Σ_{16}PAH at the highest temperatures studied (750 and 1000 °C) were always lower than values of the other temperatures. Other interesting considerations that can be made concern single PAHs trapped on biochar at 350, 550 and 750 °C. For instance, all 16 PAHs were present at 550 °C except acenaphthene, and naphthalene and phenanthrene were found to be the most common and abundant PAHs. The process (pyrolysis or gasification) influences on the concentration of PAHs in biochar, however, the design of the reactor used and the working temperature are also important. The fact that the produced gas passes through or not the char bed to exit from reactor is an important factor. The lab. pyrolysis, in which the produced gas is continuously brought away by inert gas, gave biochar with Σ_{16}PAH lower than pyro-gasification, in which the syngas passes through the char bed. For example, Σ_{16}PAH were 455 and 3989 µg/kg for lab. pyrolysis and ind. pyro-gasification biochars, respectively. In this study the ind. pyro-gasification biochars were generally characterized by PAHs that did not include those with MW > 228 uma, unlike the biochar from ind. pyrolysis that contained 15 of the 16 US EPA PAHs. Considering the TEC (toxic equivalent concentration) values, the ind. pyro-gasification biochars investigated are suitable for their potential use as soil amendments.

In conclusion, it can be said that to produce biochar with lower PAH content is necessary choose the suitable parameters concerning the feedstock, temperature, process and design of the reactor.

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Particulate and gaseous indoor contamination at the Museum of "Last Supper" of Leonardo Da Vinci: results from one year of monitoring activity

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Atmospheric particles and gaseous pollutants can affect cultural heritage, especially the most fragile, such as The Last Supper of Leonardo da Vinci. During the centuries, The Last Supper became a case in establishing the extent of the issue of environmental conditions for the preservation of the Leonardo's masterpiece. The Leonardo's painting is protected by a filtering system[1]. Previous indoor studies in the Last Supper museum were aimed to the indoor/outdoor penetration of particulate matter[2]. Uncertainties concerning the particles dynamics and the gaseous contamination still persist. At this purpose, a specific experimental setting was developed allowing to perform continuous, high-time resolution, monitoring activity: a novelty in the field of conservation of cultural heritage.

Measurements (March 2016-April 2017; 1 min time res) were conducted using a combination of: 1) Aethalometer (AE-33, Magee Scientific, 7-λ), 2) condensation particle counters (TSI 3787), 3) an optical particle counters (Grimm 1.107), 4) a low volume sampler (Mega system) 5) a custom developed rotating impactor, 6) a real-time NH_3-NO_x-O_3 detector (Environment spa) and 7) passive and active CO_2, samplers were used in order to determine the isotopic ratio $^{13}C/^{12}C$ ($\delta^{13}C$) using a newly developed method[3]. A Ca-based sorbent, $CaO/Ca_{12}Al_{14}O_{33}$, 75:25 w/w, was used for passively collecting atmospheric CO_2, and an Ascarite®-based sorbent for the active sampling; the collected CO_2 was desorbed from the substrate with acid treatment and directly analysed by non dispersive infrared spectroscopy (NDIRS).

Whereas particles concentrations reached the low level reported in previous studies[1], the high time resolution showed their diurnal cycle: the total number concentration reached a value of 20.7±0.1 cm^{-3} (BC was 8±1 ng m^{-3}) during night and then increased with visitors up to 167.8±3.3 cm^{-3} (BC was 37±4 ng m^{-3}) during daytime. These values remained however far from outdoor pollution levels ($\sim$1-2*10^4 cm^{-3} and $\sim$1000-2000 ng m^{-3}) highlighting the efficiency of the filtering system. The impact of visitors was also confirmed by CO_2 data that revealed a decreasing $\delta^{13}C$ values with increasing the number of visitors. The results on the isotopic composition of CO_2, correlated to the variation of atmospheric CO_2 concentration, permitted to follow the anthropogenic impact in the different areas of museum and were used such as a non-invasive marker to detect the air circulation and to improve the filtering system operations.

The diurnal cycle of primary and secondary particles was also investigated obtained using the apportionment of particle concentration based on N/BC ratio[4]. A secondary particles peak in the afternoon (up to 300 m^{-3}) was observed. Also the NH_3 concentration peaked in the afternoon reaching a double concentration than that observed during night-time.

Moreover, the chemical composition of collected PM samples showed a mass fraction of NH_4NO_3 of 7% inside the museum that, compared with the external value of 30% highlighted the volatilization and decomposition of NH_4NO_3. The presence of ammonia became a driver of new particle formation.

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Comunicazioni Poster

Analytical Investigation on Archaeological Patina: A Tool to Shed Light on the Provenance of Ancient Bronze Coins

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The elemental composition of archaeological patina of ancient bronze coins is very complex and it results from the deterioration processes, generically named "corrosion", that occur during the centuries. The corrosion products covering the coins are strictly correlated to the environmental characteristics of the burial site. So the analysis of coins surfaces can be a useful tool for numismatists to verify the provenance of coins: a different surface composition of similar bulk materials could be probably due to different micro-environmental conditions[1,2,3]. The aim of this study was to verify whether a set of 117 ancient bronze coins of the Roman Empire period that was declared to be found during surveys on Cesén mountain (North-eastern Italian Alps) could be compatible with another set of 103 coins coming from the archaeological excavation carried out thereafter in the same area. To avoid the damage, non-destructive analytical techniques were used to characterize the surface of six coins taken blindly from the two sets in order to highlight similarities and differences between the samples. On the other hand, some soil samples collected in the archaeological site were analysed to verify whether the patina layer of all coins could contain chemical components compatible with the elemental composition of soil. The elemental analysis of the patinas was carried out by micro-X-ray fluorescence spectroscopy (μ-XRF). To better observe the morphology of corroded surface, scanning electron microscopy coupled to energy dispersive spectroscopy (SEM-EDS) was used. Then corrosion compounds covering the coins were identified recording ATR-FTIR (attenuated total reflectance-Fourier transform infrared spectroscopy) spectra. Soil samples were analysed without pre-treatment by means of XRF and, after acid digestion, by means of inductively coupled plasma atomic emission spectroscopy (ICP-AES). The bulk composition of coins was specific of different coinage periods, whereas some elements unexpected in the metal alloy were found only on the surface of two coins, in particular Ti and Mn that were also found in soil samples. This result, together with the morpho-chemical investigation, lead us to subdivide the six coins into two groups, each containing two and four coins: this grouping corresponded respectively to the set of coins coming from the archaeological site and the set of survey. The group of four coins seems not to show the characteristics of coins exposed to soil for long periods of time: patina and corrosion products could suggest a different provenance (restored finds or even a hoard). Analytical results of this study support numismatists who were not convinced of considering all coins coming from the same site on the basis of archaeological documentations. For this reason, they will not take into account all the coins to develop interpretive hypotheses but only those coming from archaeological excavations.

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Characterization of Brass Alloys After Different Cleaning Methods by XPS and XAES.

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In the frame of the projects on the sustainability in cultural heritage[1,2], an interdisciplinary methodology has been developed for the evaluation and monitoring of the corrosion state of the inside of historical brass instruments before and after being played. There is the need to establish a protocol for preventing the damage of the instruments.

During their usage, the inner walls of the instruments get in contact with humid air, sweat and saliva. A contamination layer and a thin oxide film or a layer of corrosion product, called patina, is formed. As the acoustic properties of the instruments depend on the material composition the patina and corrosion products may compromise the quality of the sound. Thus, the brass surface has to be cleaned without changing the performance of the instruments. The aim of this work is to compare different ways to remove the patina of ancient brass musical instruments. A series of brass model alloys with a zinc content ranging between 18 and 37 wt% and some pieces obtained from old destroyed instruments, supplied by the Bern museum, have been characterized by X-ray photoelectron spectroscopy (XPS) and X-ray excited Auger electron spectroscopy (XAES) before and following the surface cleaning i.e. after mechanical polishing, cleaning with Metarex®, ultrasonic cleaning in distilled water and cleaning with organic solvents (butan-2-one, propan-2-one and ethanol). The chemical state identification and the quantification of copper and zinc have been obtained on the basis of the binding and the kinetic values of the Cu2p, $CuL_3M_{45}M_{45}$, Zn2p and $ZnL_3M_{45}M_{45}$ peaks. The results showed that on the surface of as received samples as well as after washing with distilled water, no signals from metallic Cu and Zn were observed, thus indicating that the oxide and the organic layer were thicker than the XPS sampling depth[3]. Based on the assignment of the Wagner chemical state plot[4] $Cu(OH)_2$, Cu_2O and $Zn(OH)_2$, probably in the form of hydrozincite, were identified. After cleaning with organic solvents, the intensity of the C1s peak decreased and the metallic components of Cu and Zn signals were revealed. The line shapes of Cu2p and Zn2p signals were similar to those found on mechanically polished brasses. Cleaning with organic solvents did not affect the oxide composition. Cu_2O and ZnO are found to be the main compounds of the outermost layer of the mechanically polished brasses. After cleaning with Metarex® only a very thin zinc oxide layer together with an organic hydrophobic layer of about 3 nm thickness was detected. The analytical approach used during this work allowed the identification and the quantification of the species present at the surface of the brass alloys after different surface treatments and all the results will be presented and discussed.

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Elucidation of Rituals and Food in the 'Cult Area' of Roca Through Chemical Analyses

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The application of analytical chemistry to archaeological research has increased substantially over the last half-century and today represents a major methodological subfield within archaeological science[1,2]. The paper reports the results obtained onto a selection of Final Bronze Age ceramic materials from Bronze Age site of Roca.

Two *pithoi* in fine fabric -*figulina*- and five vessels in coarse fabric -*impasto*- (one *olla*, one dipper, three small cups) have been selected and analyzed. Regarding the archaeological contexts of provenience, one *pithos* comes from the so called *Capanna-Magazzino* located in SAS VI and all the other materials come from the South-East sector of the so called *Capanna-Tempio* located in SAS IX.

Using high temperature gas chromatography and compound specific isotopic ratio, it has been possible to identify different types of lipids absorbed by the ceramic core[3]. The *pithos* from the *Capanna-Tempio* shows residues of lipids clearly identified as olive oil. The *olla* and the dipper show residues of lipids referable to non-ruminant animals. One of the three small cups does not contain any traces of lipids but quantities of beeswax and some evidence of conifer's resins. Two cups contain residues of animal lipids that can be attributed to ruminant animals, particularly to sheep.

The *pithos* from the *Capanna-Tempio* demonstrates processes of accumulation of huge quantities of olive oil in a ritual context characterized by precise comparisons with the Bronze Age Aegean. The traces of resin in the *pithos* from the *Capanna-Magazzino* could be interpreted both as results of surface treatment or as residues of various contents different from olive oil, such as flavored wine. The animal lipids found in the *olla* seem to confirm the use of this shape in food-preparing activities.
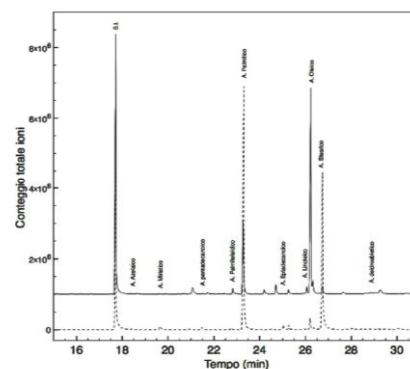
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Development of Chromatography–Atmospheric Pressure Ionization–Mass Spectrometry Methods for the Simultaneous Analysis of Different Lipid Classes Within Archaeological Finds

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The ability of our ancestors to fulfil their basic nutritional needs was a factor that shaped the evolution of modern man and the societies that followed. There is an increasing amount of evidence showing that organic residue analysis (ORA) is able to address many archaeological questions about past diets, ancient practices, early use of natural substances, but also vessel production and related use[1]. Most of the organic residue analysis have been performed on absorbed material recovered from archaeological finds, like pots and vessels[2]. Ceramic materials represent an ideal environment for the survival of residual organic matter, because its absorption into the microporous structure prevents from long-term degradation. In fact, some investigations have shown that possible contamination arising from migration of soil components, or compositional alterations through microbial activity, are minimal for compounds absorbed in potsherds[3]. Hence, these residues are a direct reflection of the original contents and usage of ceramic vessels. On these basis, this project aim is to develop chromatographic/mass spectrometry based analytical methodologies to identify the organic pyrolysis products that reside within pots used for food storage, or within hearths following the cooking of different foodstuffs. Different instrumental approaches and protocols have been developed to determine the optimal chromatograhic and/or mass spectrometric approaches to study a range of expected lipid families. These include fatty acids (FAs), fatty acid methyl esters (FAMEs), triacylglycerides (TAGs), sterols and stanols. The selective ionisation processes offered by different atmospheric pressure ionisation techniques have been utilised and shotgun analysis using ultrahigh resolution mass spectrometry methods have been compared with modern LC-MS approaches (UHPLC-MS and UHPSFC-MS). Initially, standard compounds and compound mixtures have been used, including addition of surrogate matrices representing the composition of the actual samples. The developed methods proved to be accurate (bias%<15%), precise (CV%<10%) and with high detectability (down to 1 ng/ml), thus suitable for the analysis of trace level compounds in archeological matrices. The combined use of the SIR detection mode and the high resolution ensured high selectivity and the possibility to discriminate isobaric compounds, species having the same retention time but different m/z ratio and identify molecules whose the corresponding analytical standard is not available. These protocols have been tested successively for the analysis of residual organic materials produced from a cooking hearth, with different animal/vegetable products, or present within pots. The final aim was to identify food/diet related chemicals whose qualitative and quantitative composition might represent a biomarker able to provide information related to past human activity.

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SWIR Imaging for Cultural Heritage: An Innovative Spectroscopic Method for the Study of Bronze Artworks

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In the past decades, infrared reflectance spectroscopy has been largely applied in analytical investigations of heritage materials. In particular, the production of compact systems allowed the establishment of the technique as one of the most suitable approaches for non-invasive studies on artworks by using both the mid-infrared (MIR, 4000-400 cm^{-1}) and the near-infrared (NIR, 10,000-4000 cm^{-1}) ranges. However, drawbacks related to the distortion of bands in the MIR range – mainly induced by specular reflection – have been widely reported in several research works. On the other hand, spectroscopic investigations in NIR range, characterised by the presence of overtone and combination bands, offer the possibility to obtain weaker but not distorted IR signals, providing important information for the identification of artistic materials.

To deeply evaluate the applicability of advanced methods in infrared spectroscopy, based on the use of the NIR range, a new short-wave infrared (SWIR) hyperspectral imaging (HSI) system was proposed for the study of outdoor and archaeological bronzes, whose thorough characterization still represents a crucial issue in heritage science. The sensor works in the 1000-2500 nm spectral range, with line-scan mode, and a spatial resolution < 1 mm. Indeed, bronze surfaces are highly complex and instable systems. In particular, the surface of bronze artworks usually interacts with the surrounding environment and gradually develops brown and green patinas due to the formation of corrosion products. To protect the degraded external surface of bronzes, several organic materials (natural or synthetic) are often applied. As a result, the composition of the external surface of either outdoor or archaeological bronzes results highly heterogeneous. The correct identification of all the components are fundamental to define the state of conservation of metal artworks, addressing proper restoration actions.

These advanced methods were tested on outdoor bronze specimens, as well as on archaeological Chinese coins from the Northern Song Dynasty (960-1127 A.D.). Suitable chemometric methods were deployed to correctly process hyperspectral data.

The results showed as the SWIR HSI system, coupled with chemometric data processing, was able to efficiently identify degradation products (such as brochantite, malachite, silicates and azurite), as well as organic coating products, such as waxes and Incralac™. The possibility of spatially localising such compounds across sample surfaces, provided by the potential of the imaging system, resulted a feature of definite interest for both conservation and restoration purposes.

Investigation of Modern Inks in Felt-tip Pens

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Inks of felt-tip pens are used by modern artists for the realization of sketches, drawings, copies, architectural drawings and other technical designs. However, these inks are usually very sensitive to light and chemical agents and the exact knowledge of their composition may be important to define the optimal conservation treatment and/or storage conditions. So far, few studies have been addressed to the chemical characterisation of these materials[1] and often information on binders, fillers, dyes and pigments is lacking.

In this study felt tip pens (Faber-Castell) used by the French modern artist Anne Flore Cabanis and other artist pens (Stabilo) were investigated by using an integrated analytical approach. The inks have been analysed with Fourier transform infrared (FT-IR), μ-Raman and visible reflectance spectroscopy, and pyrolysis - gas chromatography - mass spectrometry (Py-GC-MS). The Raman spectra of the Faber-Castell inks clearly show the presence of phthalocyanine, dioxazine and azo pigments. The Stabilo inks, instead, contain mixtures of dyes as evidenced by thin layer chromatography (TLC). Surface-enhanced Raman spectroscopy on the TLC spots allowed to identify triarylmethane dyes[2], rhodamine B and tartrazine. (Py-) GC-MS and FTIR spectroscopy provided data on binders, solvents and additives and confirmed the identifications of the pigments and dyes.

Since the photosensitivity of this type of inks is usually very high, a preliminary investigation on accelerated ageing on some ink samples was carried out. Spectroscopic techniques were used for the monitoring of photo-degradation.

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An XPS Study of Stones Degradation Using a Reference Gravina Tuff Placed Outdoor Under Specified, Environmental Conditions

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Within a current National Smart Cities (SCN_00520) project, entitled '*Product and process innovation for maintenance, preservation and sustainable programmed restoration of Cultural Heritage*'[1], the use of XPS (X-ray Photoelectron Spectroscopy) as a surface-specific technique, in combination with complementary surface and analytical techniques, is aimed at contributing to the diagnostic phase of the project whose procedural accuracy is essential to set intervention priorities. Since deterioration of any kind, due to ageing, natural or anthropogenic factors, begins from surface reactions, XPS can be suitably employed for the ex-situ compositional characterization of degraded compounds properly scraped from surfaces. The present contribution is focussed on the study of the main degradation products forming on stones constituting artifacts/monumental buildings in the areas of cultural heritage belonging to the municipalities of the Basilicata region. In particular, it was intended to complete the surface characterization provided by combined surface techniques[2] of a reference sample, properly sized (about $1m^3$), using Gravina calcarenite stones, and placed in a site with reported coordinates in the industrial area of S. Nicola di Melfi. In the previous work, the effect of local environments on its North, South, East and West surfaces was evaluated after three and six months of exposition, revealing already incipient degradation also influenced by the exposition side[2]. Here, we show the elaboration of additional XPS data acquired over an extended period, using the same curve-fitting procedure[3], as before. The whole data set will then be statistically interpreted for attempting a better rationalization of the alteration phenomena due to concomitant ageing, natural or anthropogenic factors occurring in real situations.
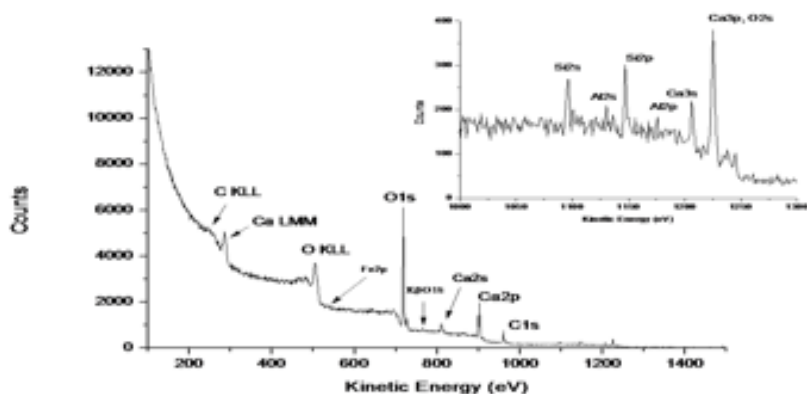
Figure 1. A wide XPS spectrum of the 'as prepared' reference sample, taken from reference 2

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Mobility of Trace Elements at the Sediment-Water Interface in Two Tourist Port Areas of the Gulf of Trieste (Northern Adriatic Sea)

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One of the main environmental issues affecting the coastal marine environments is the accumulation of contaminants in sediments and their potential mobility. The sediment acts as a sink for many chemical species (metal(oid)s, PAHs, PCBs etc.). However, this situation can be only temporary since early diagenesis biogeochemical processes can recycle these contaminants which may subsequently accumulate along the aquatic trophic chain. In this work, *in situ* experiments were conducted at two tourist ports (marinas) located in the Gulf of Trieste (northern Adriatic Sea), one in Slovenia and one in Italy. The latter is well known to be a site contaminated by long-term shipbuilding activities. The main aim was to understand if and where recycling at the sediment-water interface (SWI) may affect trace metal(oid)s. A benthic chamber, successfully used in other works in the same Gulf [1,2], was employed. Water sampling was performed during a 8-hour period to measure trace metal(oid) concentrations with time. Short sediment cores were collected near the chamber to investigate the solid (sediments) and dissolved phases (porewaters). Both diffusive and benthic fluxes were estimated to elucidate the release of trace metal(oid)s at the SWI. Total trace element concentrations were determined following a complete mineralization of the sediment (*aqua regia* + HF), whereas a weak extraction with diluted HCl (0.5 M) was carried out to quantify their potential "bioavailable fraction". Quantitative analyses were performed by ICP–OES on sediment mineralized solutions and by ICP–MS on porewater and benthic water samples. Total element contents in sediments, resulted up to two orders of magnitude higher in the Italian marina (0.4 %Pb, 0.15% As and Cu, 0.25% Zn) than in the Slovenian one, confirming the high contamination level of this site. Despite this heavy contamination, the "bioavailable fraction" of metal(oid)s extracted was scarce or null. Conversely, sediments from the Slovenian site showed high percentages of the extractable fraction from 4% (Cr) to 82% (Cu) of the total content. Trace metal vertical profiles in porewaters showed a clear diagenetic sequence. The suboxic/anoxic conditions of sediments allowed the dissolution of Fe and Mn oxy-hydroxides, thus enhancing the concentrations in porewaters of Fe, Mn and other associated trace metal(oid)s. However, the diagenetic sequence and the suboxic/anoxic transition were highly dependent on the characteristics of each site. Maximum concentrations of dissolved Fe, Mn, Pb and Zn were found in the first centimeters of one site of the Italian marina where sediments became quickly anoxic with depth. Conversely, in the Slovenian marina and in the second Italian site, the highest concentrations were recovered from the deepest porewater layers, especially for Fe (up to 2.5 and 7.7 mg L^{-1}, respectively). Positive effluxes were observed for Mn, Pb, Zn and V in the Italian marina whereas for Cu and Ni were most notably in the Slovenian marina and for as at both sites. Hg fluxes were low in spite of its high concentrations (up to 1000 mg kg^{-1}) in sediments of the Italian site. In general, benthic fluxes of metal(oid)s at the SWI can be partially explained by considering the dissolution of Fe/Mn oxy-hydroxides and metal speciation in bottom sediments. The results suggest that although the bottom sediments of the Italian marina exhibit the highest trace metal(oid) concentrations, they are scarcely remobilizable. On the contrary, in the Slovenian marina, sediments seem to be comparatively a significant source of trace elements (except for Fe, Pb and V) in the water column as consequence of diagenetic processes at the related interface.

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Iron Speciation in Antarctic Sea-Water and Sea-Ice: Significance and Analysis

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Iron is considered the most important trace element in the ocean for the growth of marine organisms and a key factor in regulating primary productivity in many areas of the world's oceans. In fact, it is involved in several metabolic pathways including biosynthesis of chlorophyll, transport of electrons through the photosynthetic and respiratory transport chains and nitrate assimilation by phytoplankton. Since marine phytoplankton can convert dissolved carbon dioxide into organic matter through photosynthesis, the iron limitation may have important implications for air-sea exchange of carbon dioxide and consequentially on global carbon and Earth's climate. This is particularly clear in remote regions like the Southern Ocean, since sea-ice contains high concentrations of iron and represents one of the most important sources of this nutrient in polar waters through melting during spring. Over 99% of the dissolved iron in the ocean is bound to organic ligands, making algal growth limited not only by a general lack of this element, but also by the bioavailability of organically bound iron.

In order to improve the understanding of iron biogeochemistry and to predict how marine productivity in polar waters can be affected by future changes, it is crucial to provide analytical methods which can detect low levels of iron in sea-water and sea-ice with high precision and accuracy, as well as to determine the amount and strength of iron-binding organic species.

In this work, we present the procedures used by our group for iron speciation in Antarctic sea-water and sea-ice. For the determination of total dissolved iron in sea-water, a low-blank pre-concentration procedure based on magnesium hydroxide co-precipitation followed by inductively coupled plasma mass spectrometry (ICP-MS) was developed. Iron speciation was determined by adsorptive cathodic stripping voltammetry (AdCSV) using a competitive ligand. This is the only technique to obtain the concentration of iron-binding ligands and their stability constant.

The described procedures were applied to samples collected during international surveys carried out in the Southern Ocean. The data collected provide paramount information on the iron sources and on the mechanisms controlling the bioavailable iron species in Antarctica.

Identification of Photodegradation Products of Alpha-Cypermetrin in Paddy Water by UHPLC-QTOF MS/MS

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Nowadays an extensive use of pesticides has been registered in order to limit specific class of pest proliferation in rice crops. This study regards αlpha-cypermethrin (α-CY), a synthetic pyrethroid insecticide, and its commercial formulation, CONTEST®, containing 14.5-15% of α-CY (w/w, water dispersible granule). Although the World Health Organization considered α-CY as moderately hazardous, other recent studies reported that neurotoxic effects could take place in humans[1]. Often degradation processes due to sunlight irradiation, hydrolysis or interactions with other compounds naturally present in paddy water, can give rise to new species that could be even more toxic than their precursors[2]. This study is focused on the natural photodegradation of α-CY and CONTEST® dissolved in paddy water, in order to better simulate their behavior in the real situations in paddy fields. Both solutions were subjected to solar box irradiation, setting temperature and intensity values to simulate sunlight conditions typical of the period in which paddy fields are submerged by water. The identification of the degradation products was achieved by a non-target approach (in Data Dependent Acquisition mode). To this purpose a new and sensitive ultra-high-performance liquid chromatography (UHPLC) coupled with a quadrupole-time of flight (QTOF) high-resolution mass spectrometry method was developed and validated. The elucidation of the unknown chemical structures of photodegradation products was carried out by using the elemental composition (tolerance <5 ppm), the relative abundance of the isotopic cluster (tolerance of 10%), the number of rings and double bonds (RDBs), the high-accurate and high-resolution MS/MS acquired spectra, from which it was possible to obtain the elemental composition for each product ion (within an average mass tolerance of 6.4 ppm). α-CY photoproducts are mainly due to the ester bond cleavage: a group of photodegradation products maintains the phenoxybenzene core, whereas another group conserves the remaining moiety of the molecule characterized by the cyclopropane core. The irradiation of α-CY in paddy water gave rise to eight degradation products, three of them have been identified for the first time, while CONTEST® solution led to nine degradation products, six of them common to those identified for the α-CY solution and two never identified before. Hydrolysis studies of α-CY in paddy water solutions, demonstrated that α-CY concentration decreases of about 63% in a month, giving rise to 3-phenoxybenzoic acid. On the contrary, CONTEST® solution showed a lower hydrolysis rate than standard α-CY, probably because of the stabilizing effect of the co-formulants present in the commercial product. Ecotoxicological tests were performed on α-CY and CONTEST® solutions, both before and after irradiation; they showed a similar toxicity of all the tested solutions. Mutagenicity of the photoproducts was evaluated through an EPA QSAR approach, by the simulation of a virtual Ames test[3]. The results were positive for three out of eleven degradation products, indicating that those degradation products are more hazardous than α-CY, hence particular attention must be paid on the use of this insecticide.

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Detection of Herbicides by Calix[6]arene-Based Fluorescent Sensors

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Diquat (DQ) and Paraquat (PQ) are non-selective contact herbicides widely used in agriculture. Numerous methods have been developed to monitor and control DQ and PQ in the environment, food, and clinical samples, including chromatography and hyphenated techniques, voltammetry and immunoassay[1]. In the last decades, many efforts have been focused on the design of supramolecular fluorescent sensors[2], which provide selectivity, sensitivity and ease of use in the detection of analytes. In the present study, we have designed calix[6]arene derivatives[3] bearing a fluorescent probe (naphtalenyl **1** or anthracenyl **2**) at the *endo* rim (Fig. 1)[4].

The recognition abilities of **1** and **2** toward PQ and DQ was first investigated by fluorescence emission spectroscopy in a solution of chloroform-methanol 1:1 in order to determine the binding constants (5). Addition of PQ to a CHCl₃/MeOH solution of calixarene (**1** or **2**) resulted in a drastic decrease of the emission intensity of the host (Fig. 2). The resulting emission spectra of **1** in the presence of PQ exhibited a quenching of the fluorescence of **1** at micromolar concentrations of PQ guest (inset in Fig. 2). The fluorescence quenching value showed a good linear relationship with PQ concentration from 4 to 120 μM (1.0 - 30.8 mg/L) with a detection lim

Fig. 1. Fluorescent calixarene hosts **1** and **2**.

μg/L, 45.5 μg/L and 25.␣␣μg/L for the PQ-**2**, DQ-**1** and DQ-**2** syst

potential method for detection of PQ and DQ by calixarene-based fluor

Fig. 2. Fluorescence quenching of **1** by increasing [PQ] in chloroform-methanol 1:1 solution at 298 K. Inset: Fluorescence change of **1** vs [PQ]
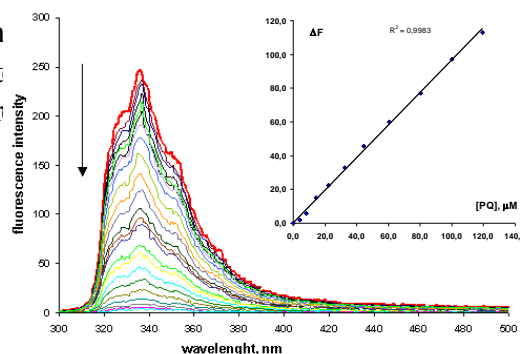
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The Use of Scallop Shell Powder as Biosorbent for the Removal of Cd^{2+} and Ni^{2+} from Natural Waters

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Intensive shellfish production generates a large volume of preconsumer and postconsumer residue, the largest proportion of which consists of shells. This material is commonly regarded as waste and its improper disposal not only results in a waste of natural resources but also can pose environmental problems. Bivalve shell is formed by biomineralization and consists mainly of $CaCO_3$ with a small amount of organic matrix, giving it a potential for use as raw material. Recycling shell waste could potentially eliminate the disposal problem, and transform a waste into high value-added products. The present study describes the use of bivalve shell, as adsorbent material for the removal of heavy metal cations from water. Heavy metals are indeed common pollutants of natural waters, especially in the proximity of mining sites and metalworking industries. In particular the adsorption behavior of two different metals is investigated: cadmium and nickel. Cadmium is toxic for humans and biota even at low concentrations, due to the fact that being a divalent cation it can substitute calcium and zinc in biological processes resulting in the alteration of cellular metabolism[1]. Because of its toxicity the limit for potable water is established at 0.003 mg/l [2]. Nickel is widely used by industries for the production of stainless steel, Ni-based alloys and Ni-based rechargeable batteries. The extensive use of nickel compounds can cause severe environmental pollution and health problems in case of exposure to high concentrations of Ni [3]. The first part of this research project was focused on the characterization of the adsorbent material, in order to determine its chemical composition and its structure and dimension using different techniques such as inductively coupled plasma, X-ray powder diffraction, scanning electron microscopy and dynamic light scattering. Batch experiments were carried out by placing in contact a given amount of shell powder with a solution containing a known concentration of metal cations. After equilibrium was reached the solution was filtered and analyzed with inductively coupled plasma atomic emission spectroscopy to quantify the amount of not adsorbed cation. Kinetic and thermodynamic properties of the biosorbent, regarding cadmium and nickel adsorption, were investigated and the kinetic equation and adsorption isotherms describing the process were obtained. The effects of some parameters as pH, temperature and salinity were also investigated to better understand the adsorption mechanism. Finally, the competition of nickel and cadmium in the adsorption onto bivalve shell powder has been evaluated.

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Analysis of the Products and Wastes of a Pyrolysis Plant Powered by Residual Plastic Materials for Energy's Production

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Alternative and renewable energy sources are going to play an increasingly strategic role in the global landscape. Among them, the waste of plastic materials produced by our society are remarkable, establishing a potential renewable source for excellence. However, it's known that recovery and recycling technologies of waste are very often expensive. When these technologies aren't workable or economically convenient, it's useful to regain the energy in the so-called "quaternary recycling", using the plastic materials as alternative fuel. In fact, the average HHV of a mixture of plastics is about 35 MJ/kg. Actually, waste of plastic materials coming from automotive industry are transferred to landfills or to incinerators, but it should evaluate the high risk of formation of oxygenated heterocyclic compounds (e.g. dioxins) as well as of all the polluting substances (e.g. polycyclic aromatic hydrocarbons) during the combustion process. Taking into account these compounds, the research has been pointed on pyrolysis equipment where a syngas is formed in an oxygen-free environment with very low emissions from the plant in the atmosphere during the process. In collaboration with MECTRA s. r. l., equipped with an innovative pyrolysis plant, it was possible to propose an analysis of its products and by-products, in order to respect its good working and to achieve a minor environmental impact according to the limits of law. Synthetically, the process allows plastic materials coming from automotive industry to be transformed into combustible gas through a slow pyrolysis at temperatures between 400 to 700 °C. Thanks to a heating induction's system, the frequency can be calibrated according to the raw material chosen. At the end of the process, a gas with high HHV is produced and used to generate electricity by an alternator linked to an endothermic engine. The aim of the research, therefore, is the physical-chemical characterization of the input materials, the syngas produced, the emission fumes from the engine, the by-products formed during the process such as carbon black, ashes, hydrocarbons in both liquid and bituminous form, and washing waters. Concerning the parameters to be monitored on input materials, it is chosen to analyze ashes, HHV, moisture content, volatile heavy metals and the presence of sulfur and chlorine. Regarding the syngas, the analysis is focused in particular on the amount of methane gas, of higher volatile hydrocarbons and eventually of HCl and CO. About the emission of pollutants from the engine the study is mainly directed to the presence of toxic compounds such as dioxins and PAHs, in order to test the actual environmental impact eventually due to the choice of raw material. In the end, the analysis of by-products is based to understand their possible toxicity and their possible reuse, analyzing the presence of numerous metals and compounds such as PAHs and PCBs. In conclusion, the analytical characterization of the above mentioned parameters is particularly important, in order to verify the versatility of the pyrolysis process in its industrial application. This characterization, in addiction, could be a starting point of future legislative measures on the environment and on the potential of residual plastics as a renewable energy resource. For future perspectives, during the testing phase, the plant is going to be fed with biomass, such as rice straw, walnut shell etc., with electrical cables, composite packaging and tire coverings.

Sito di Monitoraggio in Alta Quota nelle Dolomiti Bellunesi (Col Margherita): Sistema Informatico per la Gestione Automatizzata dei Dati Meteo-Climatici.

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L'Istituto per la Dinamica dei Processi Ambientali (IDPA) del CNR gestisce un sito di misura di alta quota (Hight Altitude Station - HAS) in un'area remota a Col Margherita. La stazione di monitoraggio, accessibile dal passo San Pellegrino e dal passo Valles, situata nelle Dolomiti bellunesi a 2530 m sul livello del mare (latitudine 46° 22' 2.11" N, longitudine 11° 48' 23.03" E), è stata installata nell'estate del 2012 nell'ambito del progetto EU - FP7 Global Mercury Observation System (GMOS).

Per risolvere le problematiche legate alla gestione a distanza degli strumenti si è resa necessaria l'adozione di specifiche tecnologie hardware-software (IT) per l'acquisizione, gestione, trasmissione e validazione dei dati forniti dalla strumentazione automatica in dotazione alla cabina. Il sistema IT è stato progettato e sviluppato per permettere il controllo degli strumenti di misura di parametri chimico-fisici, della sensoristica meteo - climatica, delle telecamere interne/esterne della stazione.

Sviluppato su architettura Gnu-Linux, è stato utilizzato principalmente software Open Source. Sono stati integrati protocolli di comunicazione seriale per il controllo degli strumenti. L'acquisizione e la validazione dei dati viene gestita con LabView e Python. La sincronizzazione e il backup sono gestiti con Bash, crontab, scp e rsync. L'analisi statistica, la visualizzazione dei dati e la reportistica automatica sfruttano gli strumenti R, Shiny, Sweave e Latex.

Sono state considerate differenti soluzioni per la conservazione dei dati in rete (Cloud Storage) e per rendere fruibili i dati ad altri gruppi di ricerca, in accordo con gli standard Open Geospatial Consortium (OGC).

Attualmente il sito di misura è dotato di strumentazione meteo - climatica, di un analizzatore di mercurio atmosferico (TGM) e di ozono ed è in grado di integrare ai dati raccolti in automatico le misure di parametri chimico fisici ottenuti da campagne di monitoraggio hot-spot (inquinanti organici e inorganici nelle deposizioni atmosferiche, particolato atmosferico e neve).

L'obiettivo è realizzare la completa automazione dei processi di acquisizione, trasmissione, visualizzazione, validazione e produzione di report automatici dei dati misurati.

Adsorption of Triazine Herbicides on Carbon Nanotube Sponges

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Because of their exceptional surface area combined with excellent chemical, mechanical and thermal stability, materials based on carbon nanotubes (CNTs) are promising candidates for the extraction of organic substances from liquid media, either apolar compounds, in the native form, or polar compounds, after CNT functionalization. The adsorption ability of CNTs has been widely investigated in solid phase extraction of solutes from aqueous or non-aqueous matrices, aimed at concentration and/or isolation of the target analytes before chromatographic analyses[1]. Several comparative studies have revealed that adsorption ability of CNTs towards environmental pollutants is similar or higher than that provided by conventional silica-based sorbents or macroporous resins. While solid phase extraction based on packed columns or filters are suitable to treat relatively small volumes of aqueous samples, large-scale decontamination processes requires dispersion of the micrometer- or sub-micrometer-sized sorbent in the contaminated water. In this condition, the disadvantages related with high back-pressure and decrease of the real surface area typical of packed columns are eliminated, but recovery of the sorbent by filtration or centrifugation is necessary to permit its successive re-utilization. CNT sponges are bulk materials consisting of self-assembled interconnected CNT skeletons with high porosity which make them promising candidates for environmental applications such as sorption, filtration and separation[2]. In this investigation, some triazine herbicides have been identified as molecular probes to test the absorption efficiency of CNT sponges towards water organic contaminants. Symetric triazines (s-triazines) have been extensively used worldwide as herbicides to control unwanted plants in agriculture and in urban areas. Because of massive use and persistence in the soils, contamination of surface and ground water by triazine herbicides and their metabolites is an emergent environmental problem in intensively cultivated areas[3]. Due to their toxicity, triazine herbicides have been included in the list of priority pesticides and carcinogenic and endocrine-disrupting activity of specific compounds have been demonstrated. Apart from their environmental and toxicological relevance, our attention was focused on triazines because their properties (solubility in water, idrophilicity/idrophobicity, and partition between organic and aqueous media) can be largely modulated by moderate changes in the substitution pattern of the triazine skeleton. In particular, the substituent pattern can influence the polarizability of π-electrons of the aromatic triazine ring and govern the efficiency of π-π interactions, regarded as the main intermolecular forces responsible for absorption on CNTs. In addition, other kinds of non-covalent intermolecular forces, such as dipolar interactions and hydrogen bonding are influenced by the degree of alkylation of the amino groups.

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Determination of Pesticides in Baby Food by Means of MEPS Followed by UHPLC-MS/MS Analysis

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Processed cereal-based baby food is an essential nutritional source for infants and young children, which are vulnerable for their poor immunologic system and low weight[1]. To protect their health the European Union Baby Food Directive 2006/141/EC designed pesticides as prohibited, and in some case their residue has a maximum residue limit /MRL) set between 3-8 µg/kg [2]. In the last years, in pesticides residue analysis, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) developed by Anastassides et *al.*[3] is the most common extraction procedure of pesticides from cereal and cereal based sample. In this work a method for the analysis of pesticides with the lowest MRLs (3-4 µg/kg) in baby food based on MEPS clean-up has been developed, also comparing MEPS with QuEChERS. Two hundred mg of sample were extracted with acetonitrile-acetate buffer (60:40 v/v); the mixture was sonicated and centrifuged for 5 min at 10,000 rpm at 20°C. An aliquot of supernatant was then subjected to the clean-up procedure using MEPS with highly cross-linked polystyrene divinylbenzene (HDVB) stationary phase. The chromatographic analysis was performed by an UHPLC coupled with triple quadrupole mass spectrometer. The acquisition window was split in three periods and quantitative analysis was carried in Multi Reaction Monitoring (MRM), selecting two precursor ion/fragment ion transitions for each analyte.
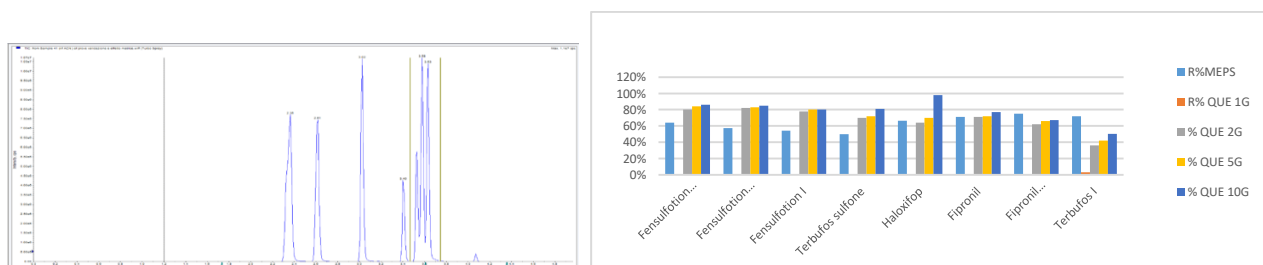
The best condition to extract all the analytes were studied: organic phase, amount of sample and extracting volume and stationary phase. After that QuEChERS was compared to our MEPS extraction procedures and we have demonstrated that we obtain better recovery an even a great enrichment factor, a reduction of time, amount of sample and organic solvent. Finally, the proposed method was validated according to SANCO/12571/2013 document: the validation data proved satisfactory reproducibility and accuracy demostrating the method is well suited to detect all analytes. In conclusion, the presented research allowed us to develop a method for the determination of several pesticides and their metabolites in baby-food at very low concentration values.

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Evaluation of the Presence of Inorganic and Organic Contaminants in Soils and Vegetables Cultivated in Urban Gardens

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In recent years, urban horticulture has had a great expansion; the principal reasons are to be sought in the possibility to produce fresh and low-cost vegetables and reduce environmental and socio-economic problems[1,2,3]. These reasons induce many cities in the world to assign common green areas to the citizens for urban horticulture. Turin is one of the Italian cities having many urban gardens thanks to "TOCC – Città da coltivare" project[4]. In order to re-evaluate abandoned green areas municipalities entrust urban lands to citizens willing to cultivate them. Nevertheless, since these areas are collocated within urban centers, both soils and vegetables grown therein are subjected to continuous pollution sources such as vehicular traffic, atmospheric pollution and contaminated irrigation waters. Moreover, the presence of both civil and industrial buildings could determine the contamination of the soil and hence the vegetables there cultivated by root uptake. The intake of vegetables contaminated by organic and/or inorganic pollutants can have serious risks for human health. For these reasons, sometimes the benefits, derived from vegetables cultivated in urban gardens, are questioned[5], and therefore urban horticulture requires further and careful studies. In this context, the aim of our study is to obtain an overview of pollution levels present in nine urban gardens, localized in Turin and in its neighboring, to understand how the anthropic activities can adversely affect these areas. For this purpose, we have determined inorganic and organic pollutants content in both soils samples and vegetables collected in these urban gardens. The elemental concentrations in soils and vegetables were determined by ICP-AES, GF-AAS and SF-ICP-MS whereas organic pollutants were analyzed by GC-MS. High concentrations of chromium and nickel have been found both in soils and in vegetables: the presence of these elements can be due to anthropic sources or, most likely, to the litogenesis of this area derived from Cr-Ni-serpentinites. This study has evidenced that the absorption capacity of heavy metals in soil depends strongly from considered plant species: lettuce and rosemary, for example, have lower concentrations of various heavy metals than the other considered vegetables (cabbage, sage, tomatoes leaves, etc.) even if they are cultivated in the same site. Indeed, we have found similar metal concentrations for the same plant species cultivated also in different urban gardens. Regarding organic pollutants, the presence of some polycyclic aromatic hydrocarbons (PAHs) was observed in the soils and in the vegetables, arising primarily from the incomplete combustion of organic materials of anthropic activities. The multivariate elaboration of experimental data has confirmed a clear difference among several plant species with regard to their inorganic and organic composition. By way of conclusion, we can suppose that in urban gardens is preferable to cultivate only specific plant species less affected by possible pollution of the area, such as, for example, lettuce and rosemary.

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Elemental Carbon, Primary and Secondary Organic Carbon in a Coastal Site in South Italy

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Carbonaceous PM is composed of a refractory component, commonly called elemental carbon (EC), and an organic fraction, called organic carbon (OC). Organic aerosols represent a large fraction of atmospheric aerosols. The organic fraction can include saturated and unsaturated aliphatic compounds, aromatic compounds, alcohols, ketones, aldehydes, carboxylic acids, amines, sugars, polyols, and organic sulfur compounds[1,2]. Organic and elemental carbon were measured in daily PM10 and PM2.5 and 6 hours range time PM2.5 samples collected from September 2015 to October 2015 in a coastal rural site near Brindisi in the Apulia region (Italy), in order to determine factors affecting the carbonaceous aerosol variations. OC and EC were measured on Sunset Laboratory Thermal–Optical Carbon Aerosol analyser using NIOSH protocol. Carbon content (total carbon TC) represented a considerable fraction for both PM10 and PM2.5. The highest values for both parameters were recorded when the winds blows from NE Europe and when Sharan Dust events have been recognized. The results show that OC and EC exhibited higher concentrations during the night hours, suggesting that stable atmosphere and lower mixing play important roles for the accumulation of air pollutants and hasten condensation or adsorption of volatile organic compounds. Moreover, ATR-FTIR analysis were performed on some samples with the lowest and the highest OCsec and on samples characterized by Saharan Dust event. FTIR spectroscopy provides absorption peaks which can be identified and assigned to chemical bonds to identify functional groups in a complex sistem. The FTIR method is relatively simple and rapid and requires only small sample size. To our knowledge, there are only few examples of recent studies which are subjected to quantification or qualification of chemical species of aerosol samples. FTIR coupled with attenuated total reflectance (ATR) is a new technique developed recently. ATR FTIR analysis were carried out and it let identify organic functional groups included non-acid organic hydroxyl C-OH group (including sugars, anhydrosugars, and polyols, herein indicated as alcohol group), aromatic C=C-H group, aliphatic unsaturated C=C-H group, aliphatic saturated C-C-H group, non-acid carbonyl C=O group, carboxylic acid COOH group, and amine NH2 group. Among the inorganic ions, carbonates, sulfate, silicate and ammonium can be recognized.

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Study of Endocrine Disrupting Compound Release from Medical Devices in Plasma Samples of Premature Newborns Through an On-line SPE UHPLC-MS/MS Method

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Endocrine disrupting compounds (EDCs) are exogenous substances that mimic and antagonize the effect of endogenous hormones. EDCs may interfere and influence the human development disrupting the synthesis and the metabolism of hormones and of their receptors[1]. Furthermore, the effects of these chemicals are not only limited to the adult life but they can also be passed on to future generations by the probable mechanism of transgenerational epigenetics inheritance[2]. Differently from adults, infants are not exposed only to chemical toxicants in the environment but they may be also exposed to EDCs indirectly during their intrauterine life. EDCs generally tend to be highly lipophilic and they can easily accumulate in fat stores inside the body. Hence, the underdeveloped reproductive apparatus and immune system during fetal and post-natal life can be exposed to unusually high concentrations of compounds that cannot be easily metabolized or excreted at these stages of development[3]. In addition, some studies reported a possible correlation between chemical exposures to EDCs of the mothers and pregnancy preterm. This study deals with the development and validation of a new analytical method for the determination of 21 selected EDCs belonging to different chemical classes (5 parabens, 2 bisphenols, 2 alkylphenols, 1 antibacterial soap, and 11 perfluorochemicals) in plasma samples. The aim was to analyze these compounds in blood samples of premature newborn patients of the Neonatology Department of Ospedale Maggiore of Novara. The analyses were carried out by UHPLC-MS/MS. The plasma samples were subjected only to protein precipitation with cold acetonitrile, centrifuged and diluted 1/10 (v/v) before purification and pre-concentration through an on-line SPE system (loading at flow-rate 3.0 mL/min for 2 min) directly interfaced with the UHPLC-MS/MS. Different SPE cartridges and chromatographic columns were tested, in order to obtain the best conditions with respect to the analytes separation, the pre-concentration factors, LODs and LOQs, injection volume and system backpressure. Starting from 200.0 µL of plasma, the LODs of the method range from 0.1 pg/mL for perfluorobutyl sulfonate to 14.9 pg/mL for bisphenol A, the recovery values being always greater than 85% and the total run time less than 9 min. The first step of the study was to identify a possible release of EDCs from the medical devices and successively the EDC accumulation in plasma of the newborns during the therapies to which infants have been subjected. Premature infants need in fact intensive medical care and they must be nourished only with parenteral solutions by tubes, intravenous catheters, and other medical devices for almost 2-3 months. Simulation of leaching on the syringes and the butterfly were carried out by using physiological solutions (3 solutions from different manufacturer) for the same contact time of the blood draws. As concerns the catheters, the simulations of release were carried out outside with the physiological solution and inside with the parenteral one for 2 week of contact time, equal to the time of permanence of the catheter into the infant body. The results show the presence of some EDCs both in physiological solution and in the medical devices commonly used during the infant hospitalization. The study is still ongoing and other plasma samples will be collected.

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Microbusters, an integrated approach for the treatment of micropollutants: the key role of suitable analytical tools

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The "Microbusters" project, funded by Compagnia di San Paolo and University of Torino, aims to develop an innovative integrated tertiary water treatment for the removal of micropollutants (MPs) such as pharmaceuticals, personal care products, etc…., from natural as well as treated waters. Wastewater treatment plants are not specifically designed nor optimized for MPs abatement at trace concentrations (from few ng/L to several μg/L). Thus, many of these MPs are still present at the end of wastewater treatment processes and can re-enter the water cycle. The presence of MPs in natural waters has been associated with many negative effects, such as short- and long- term toxicity, endocrine-disrupting effects, antibiotic resistance of microorganisms. Microbusters deals with the development of novel materials for optimizing four complementary technologies that have shown promising results in the treatment of MPs, namely membranes, adsorption, biocatalysis and photocatalysis. Moreover, based on the preliminary results, the present research has the ambitious goal of creating a consortium of academic and industrial partners for preparing a proposal under H2020, including the Life Cycle Assessment of new materials and the design of a prototype for MPs abatement in real waters at pilot plant scale. To face these aspects, beside additional expertise in the field of biology, engineering, economics, environmental and soil chemistry, the development of suitable analytical tools for MPs analysis at sub-trace level is mandatory. A list of 16 MPs, chosen on the basis of their presence in surface waters and toxicological interest, has been considered for the development of fast and sensitive UHPLC-MS/MS methods. Since the physical-chemical properties of the chosen analytes range from the high polarity of glyphosate and its degradation product AMPA to the more lipophilic alpha-cypermetrin and nonylphenol, three different analytical methods have been developed. Glyphosate and AMPA have been separated by an HILIC-MS/MS method, whereas the other two methods consist in the separation on an Eclipse XDB-C18 column (4.6x50 mm 1.8 μm, Agilent) of eight basic species (caffeine, carbamazepine, tricyclazole, diclofenac, acetaminophen, terbuthylazine, tetracycline, amoxicillin) that ionize in positive ion mode, and of five acidic species (nonylphenol, bisphenol A, butylparaben, triclosan, clofibric acid) that easily ionize in negative ion mode. All the UHPLC-MS/MS methods have been directly interfaced with on-line SPE, not only to purify the water samples, but also to pre-concentrate the MPs on the SPE HLB cartridge (2.1x20 mm, 25 μm, Waters) that is able to retain all the analytes thanks to its versatility. The analytes have been loaded on the SPE cartridge for 2 minutes at flow-rate of 2.0 mL/min in order to achieve LOD and LOQ values of the order of few ng/L. All the methods have been validated according to the UNI CEI EN ISO/IEC 17025, evaluating LODs, LOQs, linearity range, sensitivity, reproducibility, selectivity, accuracy, recovery, and matrix effect. The obtained recovery values are greater than 94%, whereas the absence of matrix effect is the result of the optimized washing step of SPE sorbent, which employs a washing volume of about 30-folds the column volume. These analytical procedures will be applied to different water samples (surface river waters, groundwater, samples taken at different stages in a water treatment plant) before and after spiking with a known amount of the 16 MPs, in order i) to perform a screening of their presence and concentration level; ii) assess the matrix effect on their determination. Afterwards the analytical procedures will be applied to evaluate the efficiency of the above-mentioned tertiary water treatments for MPs removal.

Development of Two Colorimetric Assays For Real Time Monitoring Of Saxitosin In Sea Water

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Saxitoxin (STX) is one of the most lethal non-protein toxins ($LD_{50} = 9$ µg Kg^{-1}) and is the only marine natural product that has been declared chemical weapon. STX has the ability to bio-accumulate up trophic levels. Ingestion of infected marine organisms, by humans, induces a lethal disease known as Paralytic Shellfish Poisoning (PSP) that is currently without antidote or detoxification pathway. The maximum tolerance levels, as established by the European Union and according with the Food and Drug Administration, refer to 40 - 80 µg PSP x 100 g edible portion of fresh, frozen or tinned shellfish.

The goal of this work is to develop rapid tools for monitoring the presence of STX in sea water. Two methods are proposed: a flow injection immunoassay system (FI-IA) and an Enzyme-Linked Immuno-Magnetic Colorimetric assay (ELIMC), both based on colorimetric detection.

The first method (FI-IA) consists in an off-line incubation of the sample containing STX with fixed amounts of anti-STX antibody and STX labelled with HRP until the equilibrium is established. The mixture is then injected into a flow system where the separation of the free Ag* and the antibody-bound tracer is performed in a column with immobilized protein G. In the column, all the antibodies are retained due to the protein G affinity for the constant fragment of the antibody.

The second assay is a direct competitive ELIMC where free STX and STX- HRP labeled (STX-HRP conjugate) compete towards the anti-saxitoxin antibody immobilized on the surface of magnetic beads (MBs), pre-coated with IgG anti-mouse. Separation of the unbound conjugate and the unbound free STX it is performed by capturing the MBs with a magnet and discarding the supernatant. The activity of the enzyme labelled STX is measured spectrofotometrically using the $TMB+H_2O_2$ as substrate. The optimized immune-analytical system was compared to a commercially available STX-ELISA kit.

Determination of Hydrocarbons C >12 In Soil Samples

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Hydrocarbons monitoring plays a key role to evaluate the soil quality in urbanized areas, in particular, where are present oil pumps or industrial facilities and residential areas. In recent years, the environmental concern on soils potentially contaminated by heavy hydrocarbons has increased; the current legislation focuses its attention on residential areas nearby highways or exposed to high vehicular traffic, establishing a limit value of 50 mg/Kg of total hydrocarbons. In this framework, the ISO16703:2011 method suggests to determine total hydrocarbons by GC-FID. Our study aims to optimize the proposed method in order to find the best analytical conditions to quantify hydrocarbons C>12 in soil samples. Several soil samples were collected in residential areas and close to industrial sites. Before analysis by GC-FID soil samples were spiked with different concentrations of hydrocarbons, ranging between 15 mg/kg to 75 mg/kg, and Tetracontane was added to each sample as internal standard. In order to evaluate the influence of the matrix effect on the extraction efficiency, the internal standard was added in the extraction solvent, as suggested by ISO16703:2011, or directly on the soil samples. Results from the second approach indicated a better reliability. Repeatability and other quality control parameters were investigated, confirming the good performance of the chosen method. Heavy hydrocarbons (C12-C40) were present in all samples, but samples collected close industrial areas showed an unresolved complex mixture (UCM) in the gas-chromatograms, evidence of potential oil spills.

Target and Not-target approach for the identification of pesticides and emerging contaminants in paddy water

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Rice crop requires a great amount of chemicals like fertilizers and pesticides. Nowadays, pesticides formulations are designed to offer both high selectivity and low persistence in the environment and they can undergo degradation by the effect of the environmental conditions as water, sunlight and bacteria.

According to previous studies, pesticides can potentially transform into new emerging contaminants, more toxic than the precursors[1-4], by the effect of sunlight or other environmental factors.

In this study, paddy water samples collected a rice farm located in the main Italian rice basin (north-western zone of the Padana Plain) in the North-Western zone of Italy were analyzed by the use of a micro LC system interfaced with a quadrupole-time of flight (QTOF) high resolution mass spectrometer. The quality of water was therefore evaluated according to two approaches: target and non-target.

Using the target approach, the most used pesticides in rice cultivation (like Imazamox, Oxadiazon, Pendimethalin, Profoxydim and Tryciclazol) were identified and quantified.

Regarding the non-target approach, since the well-known difficulties associated to structure elucidation and the limited availability of mass spectral libraries for LC-HiresMS, all the mass and mass/mass spectra were recorded using the SWATH[TM] (Sequential Window Acquisition of all THeoretical Mass Spectra) acquisition method. In this way, the presence of new emerging contaminants, originated from the degradation of the pesticides was investigated and their probable chemical structures were elucidated.

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Imazamox Loads In Irrigation Tailwaters And Percolation Fluxes From Paddy Fields In Northern Italy

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Italy is the Europe leading rice producer, with more than half of the total production and a high quality level. Although the main function of paddy areas is the production of rice, these complex agro-eco-systems are linked to issues of great concern such as the freshwater conservation in terms of both water consumption and water quality. An intensive experimental activity was started in 2015, in the framework of the WATPAD project (Fondazione Cariplo, Grant No 2014-1260), to investigate the water balance terms and the water quality impacts on surface water and groundwater of four paddy fields located in Lomellina (western part of the Po river plain). A different irrigation management was adopted for the four paddies: wet seeding and continuous flooding (WFL) was applied within two fields, while dry-seeding and delayed flooding was adopted for the other two paddies. The four fields were instrumented with devices measuring irrigation inflows and outflows, water level in the fields, soil moisture and groundwater depths. Additionally, in each field, three couples of porous cups were installed at depths of 25 and 60 cm. Water samples of irrigation inflows and outflows, water in porous cups, and groundwater were collected along the agricultural season (26 sampling dates in 2015). Water samples were analyzed for various compounds, including the herbicide Imazamox and its degradation products. The contemporaneous quantification of water fluxes and Imazamox concentrations allowed the determination of Imazamox loads released in surface waters and groundwater along the agricultural season for each field. The main findings, which will be discussed in the poster, are the following: *i)* most of the Imazamox loads leave the paddies through percolation; consequently, fields characterized by more permeable soils contribute more to Imazamox concentrations in groundwater; *ii)* Imazamox loads in surface outflows are modest, as the half-life of Imazamox in surface waters reached by solar radiation is very low[1]; *iii)* from the data available to date (agricultural season 2015) Imazamox concentrations in porous cups may seem to be influenced from the concomitant use of fertilizer products. In particular, it is known from the literature that the degradation of Imazamox is pH and oxygen dependent[2,3]; thus, the use of fertilizers having an effect on these water parameters could influence the Imazamox persistence in the field, favoring its transport to groundwater; *iv)* more research is needed to investigate the eventual field interaction between Imazamox and the use of fertilizers, as well as to monitor the environmental fate of Imazamox degradation products.

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Rapid PAH Determination in Beer Samples by DLLME-GC-IT/MS Protocol

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Beer is the world's most widely consumed alcoholic beverage, and is the third-most popular drink overall, after water and tea. It is thought by some to be the oldest fermented beverage. The process of making beer is known as brewing. About the main composition, beer contains phenolic acids, 4-hydroxyphenylacetic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid and sinapic acid whereas among environmental contaminants, the Polycyclic Aromatic Hydrocarbons (PAHs) are recognized as a global problem due to their chemical stability and carcinogenic effects. An Ultrasound-Vortex-Assisted Dispersive Liquid-Liquid Micro-Extraction (USVADLLME) procedure (the main steps of the procedures are reported below) coupled with a Gas Chromatography-Ion Trap Mass Spectrometry (GC-IT/MS) is proposed for fast analysis of nine Polycyclic Aromatic Hydrocarbons (fluorene, phenanthrene, anthracene, fluoranthene, pyrene, crysene, benzo(b)fluoranthene, benzo(a)pyrene and benzoperylene) in beer (alcohol by volume ≤ 7 %). Among 5 possible extraction solvents tested, dichloromethane (density 1.325 g mL^{-1} at 25 °C) was selected for further method development. Parameters such as extraction solvent type and volume, extraction time and pH, and NaCl concentration were optimized. Under optimal conditions, the enrichment factor of the nine analytes ranges between 100 and 1000 fold and the recoveries from 83 % to 99 % and correlation coefficients range from 0.998 to 0.999. The limit of detection (LOD) and limit of quantification (LOQ) are ≥ 3.8 pg µL^{-1} and ≥ 9.8 pg µL^{-1}, respectively. The precisions expressed as relative standard deviation (RSD), are ≤ 4.0 %. The entire methodology has been applied to 11 real samples (9 commercial beer samples packed by bottle and tin, and 2 special commercial beers), available in the Italian market. The only PAH present in all the samples is the chrysene, which is not relevant from a human health point of view. On the other hand, a systematic PAH presence in the samples has not been found: the only interesting issue regards the benzo(a)pyrene level in two samples. Even if it is not possible to identify the source (this should be just possible analyzing the raw materials of these two beer samples), this analysis confirms the importance of having a fast, valuable and reliable protocol for analyzing such compounds.
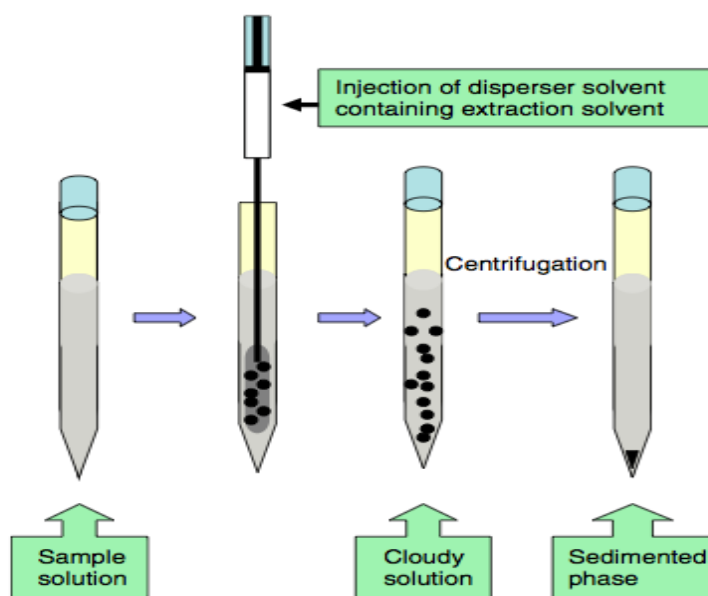
Analytical Methods to Study the Fate of Carbon in Soil Treated with Biochar

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Biochar is the carbonaceous solid obtained from pyrolysis of biomass. The addition of biochar to soils can improve fertility, meanwhile mitigating climate changes by sequestering organic carbon into a form resistant to mineralization. The porous carbonaceous structure of biochar can reduce the mobility of contaminants and improve removal efficiency due its sorptive capacity. These last properties are of particularly interest in riparian zones. However, after application, biochar is subjected to different factors that can alter its performance, like physical (vertical transport, horizontal and lateral export, slacking), biological and chemical processes. Oxidation is known to favours the degradation of the otherwise highly recalcitrant aromatic ring structures. Moreover, biochar affects the properties of soil organic matter by contributing with labile and stable polyaromatic structures[1], releasing humic-like components[2], influencing the aggregates and the soil microbial community structure. In order to understand the fate of carbon in the soil it is important to have suitable analytical tools that are not affected by the recalcitrance of the biochar. The capacity of the technique to discriminate between labile soil and stable biochar fractions is a valuable characteristic. HyPy, analytical pyrolysis in the presence of hydrogen, has been recently used to this purpose[1]. In this study, we have tested different techniques to characterise the organic carbon in reference soils treated with biochar. The biochar samples were obtained from different biomass and pyrolysis conditions, and included a biochar from a model up-draft gasifier utilised in riparian zones in field experiments. The content of inorganic and organic elemental carbon in the model soil/biochar systems was investigated by TOC analyser and CHN elemental analyser. Both techniques utilize catalyzed combustion detecting generated CO_2 by IR or TCD. Thermogravimetric analysis (TGA) was utilised to evaluate the possibility to discriminate labile (humic fraction) and stable (biochar) carbon components. The volatile matter released by TGA was characterised by analytical pyrolysis (Py-GC-MS) in order to individuate potential molecular markers of the various organic fractions of the treated soils (humic matter, biochar, sewage sludge, etc.). The preliminary results obtained from these soil model systems enabled highlighting the limits and advantages of the different techniques. TOC and CHN provided similar values with experimental variability confirming that these techniques are adequate to investigate the dynamic of carbon in soil treated with biochar. Molecular ratios (e.g. toluene/naphthalene/methylnaphthalene) from Py-GC-MS can be used to discriminate different biochar types, but failed as markers in soils for biochar with relatively low H/C ratios. Data from TGA were affected by experimental conditions and the presence of carbonates, but important information can be obtained. Acknowledgments: Study conducted within the project "Uso del biochar come filtro biologico" PSR misura 16 Focus Area 4B Regione Emilia Romagna. Sauber srl and Super Taurus srl, Az. Sperimentale Vittorio Tadini, Az. Agraria Sperimentale Stuard S.c.r.l. were acknowledged for the biochar and soil samples and helpful discussion.

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Major and Trace Elements in The Aerosol Of Central Antarctica, Dome C (Italo-French Station "Concordia")

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Major and trace elements (Al, As, Ca, Cd, Co, Cu, Fe, La, Mg, Mn, Mo, Na Ni, Pb, Ti, Zn) were determined for the first time in the aerosol collected at Dome C, Concordia Station, in the Antarctic Plateau. PM10 samples were collected during summer 2005-2006 using three high-volume samplers in two different locations: the first was very close to the station (about 50 m downwind), while the other was at the Astrophysic Tent (~800 m far upwind of the station), with two samplers installed very close to each other (sites called Astrophysic Tent 1 and 2) (1). The aims were: i) to investigate the direct impact of the station on aerosol metal concentrations and ii) to determine concentrations in a clean area, under the hypothesis of no effect of the station, according to the prevailing wind direction. The availability of the aerosol mass, which was directly measured in Antarctica by differential gravimetry, allowed us to express metal contents in terms of both mass fractions and atmospheric concentrations (1-4). Determinations were carried out by Square Wave Anodic Stripping Voltammetry (SWASV) (4) and by Sector Field Inductively Coupled Plasma Mass Spectrometry (SF-ICP-MS) (5). Results, expressed in terms of atmospheric concentrations, showed that the major constituents (Na, Ca, Mg, Al, Fe) were present in the order of ~1 ng/m^3, while for trace elements values increased from a few units of pg/m^3 (Cd, Co, As, La), to a few tens of pg/m^3 (Pb, Mo, Ti), to hundreds of pg/m^3 (Cu, Mn, Ni, Zn). In general, the highest element concentrations (e.g. Cd, Pb, Cu, Zn and As) were observed around the beginning of the expedition, for each sampling site. This can be attributed to a general contamination of the area connected to the intense activity at Concordia station, including aircraft arrivals/departures. Conversely very low values were observed subsequently especially at the Astrophysic Tent. Interestingly, when in a few days of the intermediate period the wind direction reversed with respect to the prevailing direction (i.e. from the North instead of from the South), the metal concentrations, especially of Cd and Pb, decreased at Concordia and increased at Astrophysic Tent. Principal Component Analysis allowed us to recognize three groups of elements, which were associated to typical sources, as generally recognized in the area also from measurements in snow and ice. In particular Al, La and Ti appear mainly related to crustal origin; Na, Ca, Mg, Ni, Co, Mo and Fe seem all associated to the marine source; Pb, Cd, Cu, Zn, As may be referred to the anthropic source, mainly the local human presence and activity.

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Study of As^{3+} interaction with thiols in aqueous solution

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Arsenic is an ubiquitous element present in trace in all environmental compartments and in living organisms[1]. About 60% of the arsenic is released by natural sources, as volcanic activity, but it is also due to human activities, since As compounds have numerous applications, for example in the glass and semiconductor industries, in agriculture, as pesticides and herbicide, and in many other fields[2]. Accordingly, the environmental arsenic contamination represents one of the most important problem of environmental toxicology. Its toxicity, mobility and bioavailability depends on speciation, which represents the distribution of the element amongst defined chemical species in a system. Arsenic(III) compounds, for example, are more dangerous than those containing arsenic(V) owing to the high affinity for the proteins sulfhydryl groups. A possible bond with these proteins could alter their conformation and prevent the normal interaction with other functional proteins[3]. Despite the importance of As^{3+} interactions with sulfhydryl groups, very few thermodynamic studies are reported in literature. In light of this, an investigation on As^{3+}/S-donor ligand complexation was started in aqueous solution, with particular attention to physiological conditions. In this contribution, results obtained on As^{3+}-TLA and As^{3+}-TMA systems are reported.
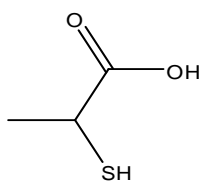
2-mercaptopropanoic acid or   2-mercaptosuccinic acid or thiomalic
thiolactic acid (*TLA*)   acid (*TMA*)

Studies were performed by different analytical techniques, such as potentiometry and spectrophotometry UV/Vis, in order to define the speciation model and all thermodynamic parameters concerning the species in solution, together with ^{1}H NMR spectroscopy in order to gain information on coordination mode.

The best speciation models obtained for the As-TLA and As-TMA systems are featured by five species: ML, MLH, ML$_2$, ML$_2$H and ML$_2$H$_3$ for As-TLA and ML, MLH$_2$, MLH, M$_2$L and MLOH for As-TMA system. For each ligand, the sequestering ability towards As(III) was determined using an empiric parameter known as pL$_{05}$, that, numerically, represents the concentration of ligand required to sequester the 0.5 of metal fraction.

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Thermodynamic Study Of Naringin Under Physiological Conditions

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Naringin and its metabolite naringenine (Scheme 1 a and b, respectively) are antioxidants of many citrus fruits.
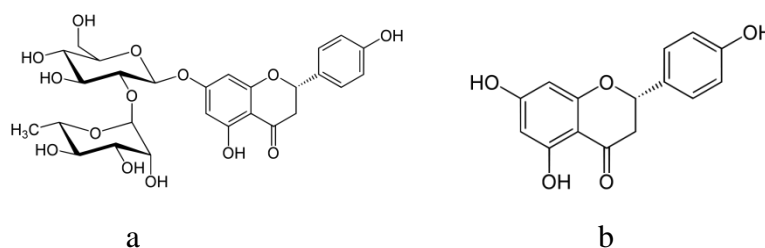
Scheme 1: Structure of naringin (a) and naringenine (b).

Naringin is the largest flavonoid present in grapefruit and has anti-ulcer, anti-inflammatory and antioxidant activity. Naringenine inhibits breast cancer proliferation and delay the genesis of breast cancer. Due to their interesting properties, naringin and naringenine have attracted the interest of several scientists in recent years[1,2].

The solubility evaluation of drugs plays an important role in the choice of the solvent suitable for the development of industrial production processes and pharmaceutical product design.

Solubility data are required to determine the most suitable solvents for extraction, separation, production and purification of organic compounds. The solubility of naringenine in the binary ethanol/water system has been reported in recent work[3]. However, comparative studies on the phenomenon of naringin and naringenine solubilities in different solvents are less studied and the information is therefore limited. Although they have a similar structure, naringin and naringenine may exhibit a substantial difference in their behavior in aqueous solution. Most real solutions show a non-ideal behaviour, so it is necessary to evaluate the effect of solvent on the analytes solubility (salting-in and salting-out). Knowledge of the analytic dissociation constants, too, may be useful for prediction of the optimal *in vivo* absorption site.

The aim of this work was to determine the solubility and the acidic constants of naringin in pure water and under physiological conditions (*i.e.* 0.16 M NaCl and 37°C).

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Thermodynamic and spectroscopic study of the interaction of Al^{3+} with olygophosphate ligands in aqueous solution

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Interest in the chemistry of aluminium complexes has increased in recent years. Compounds of aluminium occur widely in nature. Aluminium salts and compounds are toxic to animals and humans. They are involved in various disorders of the human skeleton, nervous system and blood[1]. We have studied the interaction between Al^{3+} and ortho-phosphate (PO_4^{3-}), pyro-phosphate (*PP*), tri-polyphosphate (*TPP*) and hexa-metaphosphate (*PolyP*) in aqueous solution, as these systems are relevant to both biological systems and to the environment. Models have been developed by processing potentiometric titration data to determine the stability constants of the species which are present in equilibrium mixtures. Thermodynamic parameters were obtained from data obtained with calorimetric titrations. Data were obtained at 298.15 K with a background electrolyte (NaCl) concentration in the range 0.15 - 1 mol L^{-1}. The range over which useful data could be obtained for the Al^{3+}-PO_4^{3-} system was limited by precipitate formation, even in acidic conditions. With the oligophosphates measurements could be made up to pH 9. The species AlL was found to be formed with all ligands. Other species identified include Al(LH), Al(LH$_2$), AlL$_2$ and AlL$_2$H. The complex AlL(OH), was also found with all oligophosphate ligands. Some derived numerical data are shown in the table. The parameter $pL_{0.5}$ is derived from stability constant values to provide an index of the total sequestering power of a ligand towards a metal ion[2].

Thermodynamic parameters (298.15 K, $I = 0.15$ mol L^{-1})

Ligand	PO_4^{3-}	$P_2O_7^{3-}$	$P_3O_{10}^{5-}$	$P_6O_{18}^{12-}$
$\log\beta_{AlL}$	14.07	13.53	14.11	8.42
ΔH^0_{AlL} /kJ mol^{-1}	10.2	15	25	43
$pL_{0.5}$ at pH 5	5.73	9.44	11.27	7.36

As expected for hard-hard interactions between Al^{3+} and O-donor ligands, the contribution to the standard free energy change is dominated by the entropy contribution. The chemical model for the Al^{3+}-*TPP* system and some formation constant values were confirmed with data obtained from ^{31}P NMR measurements. Chemical shift values for the individual species AlL, AlL$_2$ and AlL(OH), were calculated by means of HypNMR[3]. It appears that *TPP* is acting as a bidentate ligand. For *PP, TPP* and *PolyP* ligands, the dependence of formation constants on ionic strength over the $0.1 \leq I$ / mol $L^{-1} \leq 1$ range was calculated using a Debye-Hückel type equation.

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Study of the interaction between water-kefir grains and metals in solution

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Kefir grains are a consortium of symbiotic microorganisms, mainly consist of different lactic acid bacteria (LAB) and yeasts. The grains consist of a sugar polymer (dextran), that host the microbial consortium, and the single grains have a cauliflower like shape of 5–20 mm in diameter, a transparent appearance and an elastic structure[1]. The kefir grains ferment hexose sugars (such as sucrose) producing primarily lactic acid, ethanol and metabolites, that are released in the growth media. So, the final liquid drink, known as water-kefir too, contains many other interesting substances, among these acetic acid, glycerol, mannitol, vitamins, essential amino-acids and mineral elements. Because of nutraceutical benefits, studies concerning kefir are increasingly frequent in the last years. In addition, new researches have also showed how kefir grains are able to complex metallic cations present in the environment of fermentation but the mechanism is not fully understood. Therefore, the main goal of this study is deeper understanding of kefir complexing ability in order to assessment possible applications in health and environmental fields. To this end, the interaction between water-kefir grains and metallic ions was studied. Assuming that the proteins present on the superficial layer of the granules (S-layer proteins) are responsible of the complexing of the metallic cation, the research was initially focused into the nature of complexes, and studies of Raman spectroscopy and electronic paramagnetic resonance (EPR) were employed to investigate this aspect. Moreover, kinetic studies were carried out. The metals studied were mainly Pb, Ni, Cu, Mn, Cr(III) and Cr(VI). Pb, Ni and Cr are toxic elements, while Cu and Mn are paramagnetic elements present in natural water. In addition, other metallic species were investigated, such as Ca, K, Mg and Na. These elements are found in significant amount in the kefir grains and in water used for the water-kefir preparation. The study of the interaction between kefir grains and Cr(III) and Cr(VI) intends to understand if a different sequestering capacity towards Cr(III) and Cr(VI) exist, and if kefir grains present a reducing capacity towards Cr(VI). The studies of kinetic related to each metal were carried out putting the water-kefir colonies in sucrose and water solutions with different concentrations of single metal. The pH and metal concentrations were measured at different intervals of time and this last analysis was conducted by ICP-OES. The obtained data showed the different exchange rate of metals and the different variation of acidification rate. It can be seen that the presence, the metal type and its concentration cause metabolic interference, modifying the physiological trend of pH. This effect is more evident in the first hours after addition of the metal, afterwards to its absorption, especially at high concentrations of cations. However, the adsorption process is reversible, since the metal is partially released after long contact times (24 h) and pH reaches the physiologic value (approx. pH= 3).

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Speciation of Al^{3+} in the presence of ligands of industrial, biological and environmental interest

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This contribution is the result of an investigation on the binding and sequestering ability of three ligands different in terms of chemical point of view and of fields of application. The ligands under study are:

- Gantrez$^{®}$AN169 (H$_4$*GTZ*), a synthetic copolymer of methyl vinyl ether and maleic anhydride, having several applications in industrial, cosmetics, pharmaceutical and oral care product fields[1];
- citric acid (H$_3$*Cit*), a low molecular weight organic acid, with a well-known biological role[2];
- orthosilicic acid (*H$_4$SiO$_4$*), an inorganic compound and the main form of bioavailable silica for humans and animals[3].

Their interactions and binding ability towards Al^{3+} was investigated by performing potentiometric (ISE-H$^+$) measurements at different conditions of metal-ligand concentration ratio (c_M/c_L), temperature ($283.15 \leq T/\text{K} \leq 318.15$) and ionic strength ($0.10 \leq I/\text{mol L}^{-1} \leq 1.00$) in NaCl$_{(aq)}$, the main inorganic component of mostly natural and biological fluids. Different speciation models were determined taking into account the acid-base properties[4-7] of the ligands and of the metal cation. For all the systems the formation of a common species, namely the protonated AlLH, was observed. The investigation on the Al^{3+}/*GTZ*$^{4-}$ and Al^{3+}/*Cit*$^{3-}$ interactions showed also the formation of other species with different stoichiometry (Al$_p$$L_qH_r$), such as simple metal-ligand, polynuclear and mixed hydroxo species. The dependence of the stability constants on ionic strength and temperature was modelled by means of a Debye-Hückel type equation. From the formation enthalpy changes values, it was evidenced that all the reactions are endothermic in nature and the entropic contribution can be considered the driving force for the stability of the complexes.

Furthermore, the sequestering ability of the ligands towards Al^{3+} was investigated by the determination of an empirical parameter, the pL$_{05}$, already proposed by the research group[8]. It represents the total concentration of ligand necessary to sequester the 50% of a metal cation present in trace in solution and can be described by a sigmoid type Boltzmann equation. The analysis of the pL$_{05}$ values determined at different experimental conditions showed that in all the cases the sequestering ability increases increasing the pH and the temperature, whilst it decreases increasing the ionic strength in NaCl$_{(aq)}$.

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Interaction of 5-hydroxypyridinone derivatives with Fe(III), Ga(III), Al(III), Cu(II) and Zn(II) ions.

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The hydroxypyrones and the close congeners, hydroxypyridinones, are a particularly versatile class of ligands for biomedical use[1]. Key features of these ligands are: a six-membered ring, with a ring N or O atom either *ortho* or *para* to a ketone group, and two *ortho* exocyclic oxygen atoms. Readily functionalizable, the hydroxypyrones allow one to achieve a range of di- and trivalent metallocomplex stabilities and can include tissue or molecular targeting features by design[2]. Hydroxypyrone applications vary from metal ion removal or supplementation, contrast agents in imaging applications, to mobilization of undesirable excess of metal ions [1-3]. Four new kojic acid derivatives (Scheme 1) were synthesized and their dissociation constants as well as theit ability toward Fe(III), Ga(III), Al(III), Cu(II) and Zn(II) ions was evaluated using potentiometric, calorimetric, NMR and UV–Vis techniques. Structural information on the complex formation equilibria have been obtained from 1D and 2D NMR study. The donor atoms involved in the coordination of Fe(III), Ga(III) and Al(III), Cu(II) and Zn(II) ions are (O, O) the same at acidic and neutral pH values, even if from the complexation competition study the ligand appears to be more selective toward Fe(III) ions supporting that it can be used as an iron chelating agent. The involvement of N-donor atoms at high pH in Cu(II) and Zn(II) coordination has been determined by using UV–Vis or NMR techniques.
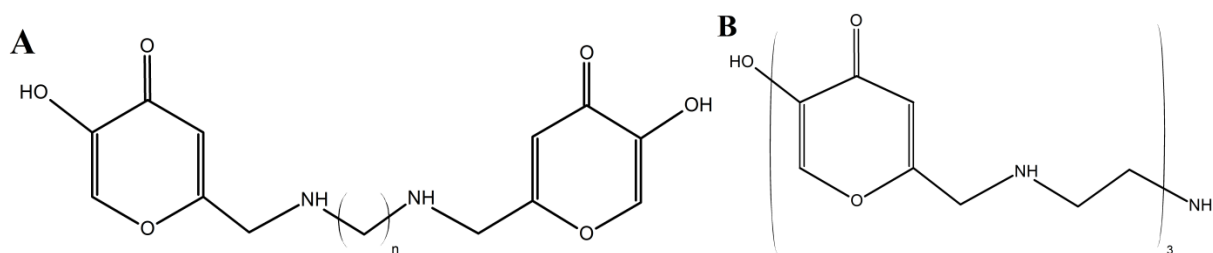
Scheme 1. Molecular structures of (A) S1 (n=2), S2 (n=3), S3 (n=4) and (B) S4.

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Survey of the solubility data of organic molecules. Classification and modeling of the factor influencing Setschenow coefficients in several ionic media.

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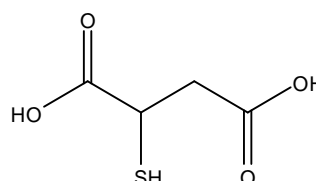Silvio Sammartano[a]*

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In this contribution, a survey of the total and specific solubility of several organic molecules is performed. Even if the importance and the impact of the knowledge of the solubility is obvious (not only for the field of analytical chemistry), the experimental determination of these parameters, and of the Setschenow coefficient[1] is often limited to few articles per year, and consequently the number of indexed scientific journals interested on these topics is generally decreasing. As an example, no database are available for this kind of information. For this purpose, a quantitative analysis of the factors influencing the solubility and the modeling of the Setschenow coefficient can be helpful to rationalize the results published in the past, to give tools to estimate the solubility of new compounds, and predict the Setschenow coefficient, the parameter that accounts for the increase (salting-in) or decrease (salting-out) of the solubility when the concentration of the background salt increases. Literature data, relative to total and specific solubility on pure water, together with Setschenow coefficients in different ionic media, were collected and critically analyzed, assigning a weight on the basis of the reliability of the experimental determination. Then, each datum was assigned to a class, based on chemical properties, such as structure (linear or cyclic), functional group (carboxylates, amines or mixtures), number of carbon atoms, values of the protonation constants etc… Once the literature data are rationalized and classified, they can be modeled using empirical equations and principal component analysis to obtain quantitative information about the descriptors of the Setschenow coefficients and the solubility in pure water. The results will be tested to check the goodness of the fit and the models will be validated splitting the sample in a test set, containing 90% of the whole data, and an evaluation set, containing the remaining 10% of the sample.

The models obtained for the estimation of the Setschenow coefficients will be compared with the existing ones, such as those of Yu et al. (based on IEF-PCM calculations)[2], Xu et al. (based on QSPR studies)[3] and Ni and Yalkowsky (based on water/octanol partition coefficient)[4].

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Modulation of acid-base properties in fused-ring n-rich aromatics

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Heterocycles are key compounds of synthetic chemistry. This class of compounds shows many relevant applications in biochemical field[1], but recently they are playing a fundamental role as building blocks of active molecules in many fields of advanced materials: conducting polymers[2], organic field-effect transistors[2], Dye-sensitized Solar Cells (DSSC)[3], nonlinear optically active and liquid crystalline compounds[4], high energy compounds[5]. The [1,2,4]triazolo[3,2-c][1,2,4]triazole system is a fused-ring N-rich aromatic system with remarkable acid-base properties and tautomerism. It is possible to exercise a fine control of the tautomeric forms present in solution or in the solid state and of the acid-base properties in the fundamental and excited state, by acting on the electronic character of the group at position 7 of the heterobicycle. In fact the relative amount of the two singly protonated tautomeric forms (1*H*-3*H* and 2*H*-3*H*) can be finely tuned in such a way that only 1*H*-3*H* is present, or only 2*H*-3*H* or both. In the case of the phenol substituent (7-p-hydroxyphenyl), both tautomeric forms are present in comparable amount in solution; the two tautomers can also be selectively precipitated in different crystalline salts using suitable counterions[6,7]. In this work, we propose a study of acid-base properties of the structural isomers of the 4-methyl-7-(hydroxyphenyl)-2H-[1,2,4]triazolo[3,2,-c][1,2,4]triazole (Fig. 1). The hydrolytic equilibria have been studied at 25 °C in constant ionic medium NaCl 0.5 M/ethanol 4% (v/v) by recording UV-VIS spectra (absorption and emission) at different pH. The experiments have been performed as acid-base titrations at constant concentration of TT5, without varying the 0.5 M level of [Cl⁻]. The pH investigated spans between 0.3 and 11. For each experimental point, the equilibrium free proton concentration was evaluated from the measured electromotive force at the ends of the galvanic cell *G.E. /T.S. /R.E.*, where *T.S.* indicates the Test Solution, *G.E.* is the glass electrode and *R.E.* is a reference electrode.
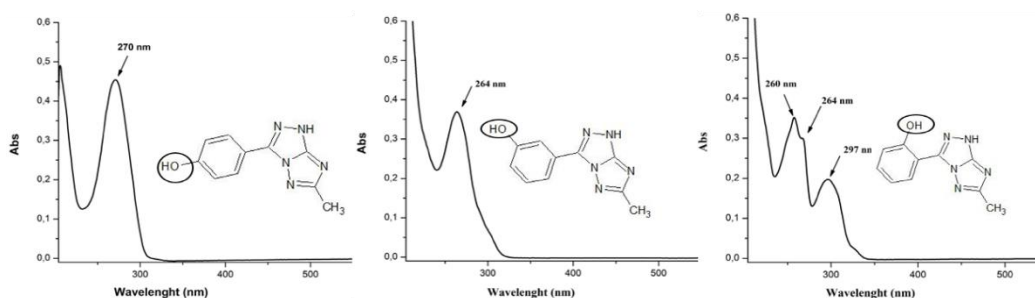
Figura 2 UV- VIS absorption spectra of three structural isomers register in NaCl 0.5M/ethanol 4% (v/v) at pH 4

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Oxidized G-C$_3$N$_4$ For the Photocatalytic H$_2$ Production From Glucose Aqueous Solution Under Solar Light

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In the past few years we have studied the application of various (waste) biomasses as the sacrificial agents to assist the photocatalytic H$_2$ production from water, using the well known Evonik P25 TiO$_2$, modified with Pt (1,2,3). In this work, we explored the photocatalytic behaviour of graphitic carbon nitride (g-C$_3$N$_4$), a novel organic semiconductor that is attracting great interest in view of the low cost preparation and its unique properties, such as visible light absorption (band gap 2.7 eV), high surface area 2D structure, chemical stability, and stronger reduction capability of photo-induced electrons compared to TiO$_2$ (4). The g-C$_3$N$_4$ catalyst was prepared by thermal condensation of dicyandiamide (500/650°C, 4 h, N$_2$ flow), then it was refluxed in 7.35 M HNO$_3$ solution under magnetic stirring (3 h) to obtain the oxidized material (o-g-C$_3$N$_4$). Both materials were characterized by X-ray powder diffraction (XRPD), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR) and diffuse reflectance UV-Vis spectroscopy (DRS), and they were tested in aqueous solution in presence of glucose as biomass-representative sacrificial agent, using Pt as the co-catalyst by in situ photodeposition. Lab-scale experiments were carried out to investigate the variables potentially affecting H$_2$ evolution from various natural waters, that is catalyst amount, Pt loading, glucose concentration. Irradiation was performed on 28 mL capacity Pyrex glass containers, under simulated solar light (500 W/m^2, outdoor filter) and magnetic stirring (6 h). The evolved H$_2$ was quantified by headspace gas chromatographic analysis (TCD detector, Ar carrier gas). Considering the 2D structure of the catalyst, irradiation experiments were performed after sonication to enlarge the available surface area and to favor its suspension in the aqueous sample. Preliminary tests in distilled water indicated that o-g-C$_3$N$_4$ has a higher photocatalytic activity (about 20-fold) for H$_2$ production compared to g-C$_3$N$_4$, not further investigated. H$_2$ evolution from water was strongly enhanced by addition of glucose compared to pure water (0.2% from direct water splitting), while no H$_2$ was detected omitting the catalyst and/or the co-catalyst (as expectable). These results clearly show that o-g-C$_3$N$_4$ is effectively able to assist H$_2$ production from water under simulated solar light, in presence of a sacrificial biomass. The evolution profiles proved to be strictly dependant on the experimental conditions, with reaction yields favored by increasing glucose concentration and Pt loading, up to 0.1 M and 3 wt%, respectively. With regard catalyst concentration, H$_2$ production was strongly improved working with small amount of o-g-C$_3$N$_4$, that is 0.25 g/L. Under these conditions, H$_2$ yields up to 840 µmol/h per gram of catalyst were achieved, and further work will be undertaken testing environmental waters (river and sea waters), under natural sunlight (outdoor conditions). The results obtained from application of o-g-C$_3$N$_4$ are then critically compared to those observed using platinized commercial TiO$_2$, chosen as the reference catalyst. Additional characterizations will be performed (by BET surface area measures and X-ray photoelectron spectroscopy, XPS) in order to correlate the physical-chemical properties of the catalysts to the performance for H$_2$ photoproduction.

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Photolytic and photocatalytic degradation of Glucocorticoids in water under simulated solar light

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Glucocorticoids (GCs) are anti-inflammatory drugs employed in human and veterinary medicines, sometimes as growth promoter. They are partially metabolized in hepatic and extrahepatic sites, and excreted by the kidney[1]. Their occurrence has been assessed in the aquatic compartment ranging from a few ng L^{-1} up to some tens of ng L^{-1} [2]. This indicates their ineffective removal by conventional wastewater treatment plants. Photodegradation is a significant tool for the abatement of persistent pollutants in water system. The photodegradation of some among the most frequently prescribed Glucocorticoids (Betamethasone, Cortisone, Dexamethasone, Hydrocortisone, Prednisolone, Prednisone, Triamcinolone acetonide) was investigated in tap water under simulated solar light, also in the presence of suspended TiO_2. The experiments were carried out in a lab-scale batch reactor at environment-significant concentration (50 µg L^{-1}) and at mg L^{-1} levels. Irradiated samples were analyzed by HPLC with electrospray ionization tandem mass spectrometry detector (HPLC-ESI-MS/MS) and by HPLC with UV detector, respectively. A first order kinetic was obeyed upon both direct photolysis and heterogeneous photocatalysis. The photoproduced intermediates profiles were followed by HPLC-ESI-MS/MS, after extraction with CH_2Cl_2 and evaporation of the eluate. For example, in Figure 1a and 1b the decomposition profiles of Dexamethasone under direct and TiO_2-promoted photolysis and the photoproducts profiles from direct photolysis are shown. Degradation rates were markedly faster by heterogeneous photocatalysis for all the drugs. This supports the contention that TiO_2 photocatalysis under simulated solar light is a convenient and efficient method for the remediation of pollutants. Photoproducts from both photolysis and photocatalysis will be identified to better clarify photodegradation p
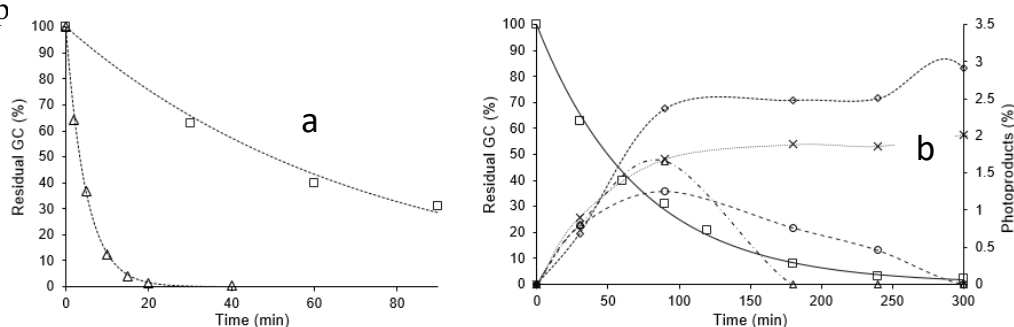
Fig.1. (a) Decomposition profiles of Dexamethasone (10 mg L^{-1}, tap water, simulated solar light) under direct (□) and TiO_2-promoted photolysis (△); (b) Dexamethasone (10 mg L^{-1}, tap water, simulated solar light) photoproducts profiles from direct photolysis.

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Determination of Volatile Organic Compounds in Exhaled Breath of Heart Failure Patients by Needle Trap Micro-Extraction Coupled to GC-MS/MS

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An innovative approach based on Needle Trap Micro-Extraction coupled to GC-MS/MS technique was developed and used to monitor Volatile Organic Compounds (VOCs) in exhaled breath of Heart Failure (HF) patients. HF is a complex clinical syndrome caused by a structural and/or functional cardiac abnormality. Patients hospitalized for HF are at high risk for re-hospitalization principally due to exacerbation of symptoms or signs of HF. Thus, a regular check of biomedical parameters is recommended for a suitable patient monitoring. With respect to the conventional clinical investigations (dosage of natriuretic peptides in serum, e.g. B-type or N-terminal pro-B-type natriuretic peptides, and transthoracic Doppler 2D echocardiography), breath analysis is an extraordinarily attractive approach for a most effective, easy, painless and non-invasive monitoring of patients suffering from HF. In the present work, the innovative and emerging technique based on Needle Trap Micro-Extraction was applied to determine VOCs in exhaled breath of HF patients. Needle Trap Devices (internal diameter 0.34 mm and length 6 cm) packed with 1 cm of Divinylbenzene, 1 cm of Carbopack X and 1 cm of Carboxen 1000 were used to collect and pre-concentrate VOCs in a single step. The Needle Trap Devices were then introduced in the injection system and VOCs were thermally desorbed and analysed by GC-MS/MS. The analytical figures of merit are discussed and the results obtained on 30 HF patients are presented.

Tests on organic pollutants: DNA/BSA binding and micellar retention of two model PAHs and two model pesticides

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Organic pollutants as polycyclic aromatic hydrocarbons (PAH) and pesticides are nowadays ubiquitous[1]. PAH and pesticides are highly lipophilic and all at least suspected of carcinogenic effects[2,3]. Information on their toxic activity is often related to *in vivo* studies, whereas a detailed analysis of the mechanistic/chemical aspects of the binding to biosubstrates is sometimes missing. To contribute to this field and in the frame of our participation to PNRA (National Antarctic Research Program) we have carried out some tests on the binding of two PAH and two pesticides (Figure 1) to biosubstrates as natural DNA and the protein bovine serum albumin (BSA). The interaction with micelles and liposomes was also tested, both as to scale the lipophilicity of these species and to get information of the possible accumulation on membranes.
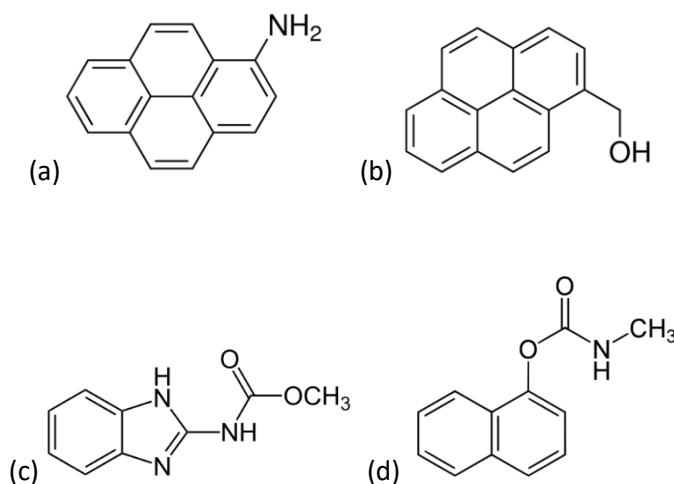
Figure 1. The PAH and pesticides tested: (a) 1-aminopyrene, (b)1-hydroxymetilpyrene, (c) carbendazim and (d) carbaryl.

The results collected show that the high hydrophobicity of these species turns into very high affinity for DNA. Absorbance and fluorescence titrations suggest complex binding modes that are discussed in relation with the different pollutant/DNA ratio. BSA binding is also found to occur in the case of the two PAH. Ultrafiltration coupled with absorbance spectroscopy enables the percentage of retention (R%) on the micelle/liposome be measured. R% dependence on the molecule and on the type of system (sodium dodecyl sulphate anionic micelles, TritonX-100 neutral micelles, dodecyl trimethyl ammonium chloride positive micelles and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine liposomes) is discussed.

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Analysis of Neurotoxin BMAA by Liquid Chromatography-High Resolution Mass Spectrometry: Over the Misidentification

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Since 1940's, the consumption of cycad seeds was associated with the increased incidence of amyotrophic lateral sclerosis (ALS) and Parkinson-like disease in the population of the Guam isles. The neurotoxin β-N-methylamino-L-alanine (BMAA), isolated from the cycad kernels, was hereafter suspected to be involved in these neurological diseases. Actually, this amino acid was produced by a cyanobacteria living in symbiosis with the cycad roots, and was then reported as a metabolite of various cyanobacterial species, so that its presence in algae and the consequent bioaccumulation in derived-food were considered to play a relevant role in such diseases. Anyway, the widespread reports of BMAA were impaired by analytical false-positives due to the isomeric amino acid α-,γ-diaminobutyric acid (DAB)[1]. Figure 1 shows the structures of the two compounds.
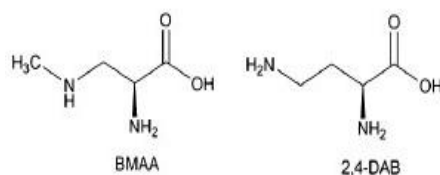
Figure 1: structures of BMAA and its isomer α-, γ-diaminobutyric acid (DAB)

As a matter of fact, the use of mass spectrometric (MS)-based methods did not eliminate the possibility of an overestimation of the neurotoxin concentration, since the simultaneous presence of two isomeric hydrophilic compounds hindered the use of the conventional reversed phase chromatographic separation.

A method based on liquid chromatography coupled to high resolution tandem mass spectrometry (LC-HRMS/MS) able to reliably quantify BMAA was developed. Specific fragment ions were highlighted for BMAA and DAB. After the evaluation of different chromatographic strategies, a satisfactory separation of BMAA from DAB was accomplished by using a mixed cationic-reverse phase column (Figure 2).

The method was validated in two different matrixes, i.e. water and plasma samples, in order to propose an analytical tool for monitoring occurrence of BMAA in environment and its possible distribution in biological samples. The analyses of BMAA in extremophile cyanobacteria typically produced in hot springs from Abano (Padova, Italy) and in the Antarctic region were also performed.

Figure 2: LC-MS chromatogram related to the final experimental condition for analyzing BMAA.

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Thallium Stimulates Ethanol Production in Immortalized Hippocampal Neurons/Cell Line

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Thallium (Tl), is a soft metal and the oxidation states are I and III. Thallium is more toxic to humans than mercury, cadmium, lead, copper, or zinc, and is responsible for many accidental, occupational, deliberate, and therapeutic poisonings since its discovery in 1861[1].

Mechanistically thallium is known to bind to sulfhydryl groups of proteins and mitochondrial membrane, thereby interfering with a range of enzyme reactions (the vital potassium-dependent processes, substitution of potassium in the (Na^+/K^+)-ATPase, pyruvate kinase, ATPase, aldehyde dehydrogenase)[2] and leading to a generalized poisoning including ligand formation with protein sulfhydryl groups and inhibition of cellular respiration[3,4]. Since Tl^+ and K^+ are both univalent ions with similar ionic radii, Tl is able to interfere with potassium-dependent processes, and then mimics potassium in its movement and intracellular accumulation in mammals[5]. Even if the neuronal Tl toxicity is well known[6,7], at the present, no few experimental data are available on Tl-induced alterations of neuronal metabolism evaluated with HS-GC-MS or HPLC analytical techniques. In this work, we present the results of such an analysis on immortalized hippocampal neurons (HN9.10e cell line). We chose to investigate Tl effects on this neuronal cell line since it shares a large number of structural and functional features with primary hippocampal neurons and, consequently, it is a reliable *in vitro* model of one of the most vulnerable regions of central nervous system, particularly susceptible to the toxic action of drugs or poisons and also severely damaged by neurodegenerative diseases. By HS-GCMS and HPLC analysis, we examined both the acute and the long-term effects of Tl exposure, finding remarkable and unexpected variations in metabolite composition of cell culture medium (CCM) variations in volatile organic compounds (VOCs) released in CCM. The increase of lactate and EtOH in Tl-exposed cell cultures suggests significant alterations in energy metabolism[8]. We would suggest in HN9.10e cells the activation of an ancestral mechanism to survive hypoxia/mitochondrial impairment, a kind of "functional hypoxia", in which the EtOH production is the key adaptation that allows a continued high level of glycolysis[9].

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Fluorescent Core-Shell Molecularly Imprinted Nanoparticles for Detection of Sphingosine 1-Phosphate

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Sphingosine 1-Phosphate (S1P) is a biologically active lipid that plays a crucial role in several cellular processes such as growth, proliferation, motility and apoptosis. It is known that high S1P concentrations in the circulation (blood and lymphatic fluid) is correlated with cancer growth[1]. This highlights the need for selective and sensitive means to detect this biomarker for the diagnosis of tumors. Presently, the most used detection methods are based on HPLC separation of fluorophore-modified S1P and LC-MS/MS, procedures that require time consuming steps and expensive instruments. In this context, the concept of fluorescent molecularly imprinted polymers (MIPs)[2] can be very useful to develop a selective, sensitive, fast and low-cost detection method for S1P. We here report a molecular imprinting strategy for the detection of S1P based on MIPs, prepared using fluorescent monomers capable of reporting binding events real time. The MIP was characterized by FT-IR, SEM and DLS. Preliminary fluorescence tests show greater selectivity of MIP for S1P in comparison to the template and other molecules containing phosphate group. Further investigation of the recognition behaviour will be carried out in different solvents, with different ratios, in order to evaluate a possible application with real samples, such as human serum. For the same purpose, X-ray Photoelectron Spectroscopy (XPS) analysis will be performed to obtain useful information about the interactions driving lipid recognition in these polymers.

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Extraction of UV Filters from Water Samples by Dispersive Solid Phase Extraction Using a New Magnetic Graphitized Carbon Material

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Magnetic solid phase extraction (mSPE) is one of the most promising new extraction methods for liquid samples before ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis[1]. In mSPE, a composite material is constituted by a magnetic core covered by an organic or inorganic coating. In addition, some carbonaceous materials, such as activated carbon[2,3], carbon nanofibers[4], and multi-walled carbon nanotubes[5] were also modified by deposition of Fe_3O_4 onto the carbon material structure itself. Graphitized carbon blacks (GCBs) are a group of carbonaceous materials made from carbon blacks by heating them in an inert atmosphere, at T>2500 °C, which differ from one another by the surface area. GCB is considered to have homogeneous non-porous graphitic surface essentially oxygen-free giving, therefore, only van der Walls and π-π interactions. However, it has been demonstrated that a relatively small-portion of the surface is still contaminated by oxygen bound to graphitic crystallite edges[6]. In the present research, for the first time, the preparation, characterization and sorption capability of Fe_3O_4-GCB composite (mGCB) toward some compounds of environmental interest were investigated. First, GCB was oxidized with nitric acid, this resulting in a mild oxidation of some oxygenated heterogeneities already present on GCB surface. Then, the material was magnetized by a simple solvothermal synthesis of Fe_3O_4 on its surface. The magnetic composite showed a surface area of 55 $m^2 \ g^{-1}$ and 55% of Fe_3O_4. The prepared mGCB was firstly tested as an adsorbent for the extraction from surface water of fifty pollutants, including estrogens, perfluoroalkyl compounds, UV filters and quinolones. The material showed good affinity to many of the tested compounds, except carboxylates and glucoronates; however, some compounds were difficult to desorb. Ten UV filters belonging to the chemical classes of benzophenones (seven) and p-aminobenzoates (three) were selected, and a method for determining these compounds in surface water by mGCB extraction followed by UHPLC-MS/MS analysis was validated and applied to eight surface water samples. The method was validated in terms of linearity, trueness, intra-laboratory precision and detection and quantification limits. In summary, the method performance (trueness, expressed as analytical recovery, 85-114%; RSD 5-15%) appears suitable for the determination of the selected compounds at level of 10-100 ng L^{-1}, with detection limits in the range of 1-5 ng L^{-1}. Finally, the new method was compared with a published one, based on conventional SPE with GCB, showing similar performance in real sample analysis. To the best of our knowledge, mGCB stable in aqueous medium has never been prepared, characterized and used to extract pollutants from environmental waters.

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Ultrasound-Assisted Emulsification Microextraction for Analytical Determination

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Ultrasound spectroscopy is a suitable tool for preparation and determination of food emulsion during processing. For such systems, a key parameter is the dispersed phase volume fraction. The dispersed volume fraction determines the relative proportion of oil and water in a product and of course has a pivotal role in the prediction of volumes during a microextraction process for analytical determination. An Ultrasound-Vortex-Assisted Dispersive Liquid-Liquid Micro-extraction procedure coupled with Gas Chromatography-Flame Ionization Detector is proposed for a reliable and sensitive analysis of phthalate esters in tea beverages[1]. Phthalates esters can be found in a wide range of products, including adhesives and glues, tubing, and food packaging. These compounds are easily released into the environment, as they are not covalently bound to the mixtures where they are used, in particular in hot drinks. Phthalate esters were extracted from samples (fresh prepared infusions of coffee and tea), using small amounts of CH_2Cl_2 (extraction solvent)[2]. The solution was vortexed, then kept in an ultrasound bath (40 kHz of ultrasound frequency and 110 W for 10 min at room temperature), finally the sample was centrifuged at 5000 rpm for several minutes. The bottom phase of the chlorinated solvent was separated from the liquid matrix and the determination of PAEs was performed by a GC Fid. An Enrichment Factor of about 150-200 times with a good recovery was found. The efficiency of the liquid-phase micro-extraction process was affected by the samples peculiarity, therefore the analytical characteristics of the method were evaluated in the linear range 0.5-50 ng mL^{-1} [3]. The validation of the proposed method was according to the Commission Decision EC/657/2002 concerning the performance criteria of analytical methods.
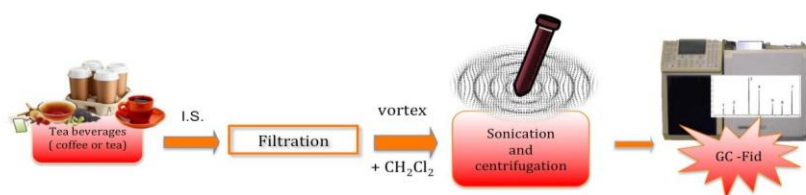
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Centrifugal and Asymmetrical Field-Flow Fractionation for the analysis of ZnO particles in cosmetic products

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Zinc oxide (ZnO) is a versatile material used in many productive sectors, of which the rubber application accounted for the largest market share, followed by ceramics, chemicals, pharmaceuticals, cosmetics, personal care and agricultural products. In the cosmetic sector, ZnO is used as a colorant or bulking agent, but mainly as UV-blocker, soothing and protective coating against skin irritation and abrasion, analogously to its use in the pharmaceutical products, mostly as ointments and creams, where it carries out antibacterial, antimicrobial, disinfecting and drying actions.

The recent increase of nano-materials to enhance the quality of many products has determined the revision of many existing international regulations, either in the cosmetic and food sectors. The European Union (EU) Commission's Working Group on Cosmetics, in 2016, for example, stated that ZnO-NPs cannot be used in spray products, or if it used in dermal application, ZnO-NPs must have a median diameter of the particle number size distribution D50 (50 % of the number below this diameter) > 30 nm and D1 (1 % below this size) > 20 nm[1].

The size analysis of ZnO particles dispersed in cosmetic or pharmaceutical formulations is consequently now an important task that analytical methods should face[2], since they could be used to discriminate the presence of particles in the nano or/and micro form. This implies that the analytical methods should be able possibly to determine the particle size distribution or at least to contribute, for example through a separation, to its determination. There are a number of analytical methods that might be used to size the particles[3,4]. In many cases, the determination of the average particle sizes and the size distribution of particles incorporated in cosmetic products, requires a preliminary extraction step. This work has consequently two main aims, to study whether and how the extraction procedures should differ depending on the cosmetic matrices and to compare the separation performances of two FFF techniques, Centrifugal FFF (CF3) and Asymmetrical Flow FFF (AF4), both used for the particle size characterization. The separation performances of CF3 and AF4 are evaluated by analysing (i) two different types of pristine ZnO powdered particles, bare and superficially modified with triethoxycaprylyl silane, available on the market to formulate cosmetic products, (ii) four cosmetic creams formulated in-house, and (iii) three cosmetic commercial products, sold in Italy and Germany for the baby skin care.

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Planar and Column High Performance Liquid Chromatography of Biologically Active Compounds Extracted from Leaves and Fruits of Cyclanthera pedata (L.) Schrab

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This communication reports the results of a recent study carried out to develop straightforward methods for the identification and quantification of phenolic compounds occurring in the leaves and fruits of *Cyclanthera pedata* Scrabs (Caigua), as well as in food supplements produced from this plant. Caigua is an ancient Peruvian plant belonging to the Cucurbitaceae family, which is grown for its edible mature fruit in many parts of Central America, South America and the Andes. It is found in the Caribbean Islands, Colombia, Bolivia, Peru and Mexico. Caigua is also an ancient medicinal plant whose use dates back to the Inca population. Nowadays, this plant is gaining market importance around the world due to its anti-inflammatory, hypoglycaemic and hypocholesterolemic properties. Recent scientific studies attribute the anti-hyperglycemic properties of Caigua to the high content of phenolic compounds, the most abundant class of secondary metabolites, and, in particular, to the glycosilated flavonoids subclass. The occurrence of phenolic compounds in the leaves and fruits of Caigua grown in Italy and Slovenia was investigated using both high performance liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC). Sample detection was performed by either photodiode array (PDA) spectroscopy or mass spectrometry (MS) with electrospray (ESI) ionization, used for obtaining structural information on the separated compounds and for their quantitative analysis. A special surface sampling probe was used for extracting the analyte on-line from the HPTLC plate to the ESI-MS instrument. The separations by HPTLC were carried out in normal phase mode using silica plates and mobile phases consisting of a variety of organic solvents in mixtures at different volume rates. HPLC was performed in reversed phase mode employing a narrow-bore C-18 column and a semimicro photodiode array detector (PDA) cell of 2.5 μL, in conjunction with a single quadrupole mass spectrometer, equipped with an electrospray ionization source (ESI-MS). The C-18 narrow bore column was eluted by a multi-segment gradient of increasing concentration of acetonitrile in water-formic acid solution that has been optimized on the basis of the results of a study carried out to evaluate the influence of mobile phase composition and gradient shape on separation performance and detection sensitivity by ESI-MS. The identification of individual phenolic compounds was performed on the basis of their HPTLC retention factor (R_f) and HPLC retention time and both UV-visible and mass spectra, acquired by PDA and ESI-MS, respectively. Different organic solvents and hydro-organic mixtures were tested for their efficiency to extract the phenolic compounds from leaves and fruits of *Cyclanthera pedata* grown either in Italy (Rome) or Slovenia (Ljubljana), as well as from commercially available food supplements. A HPTLC-DPPH method was developed in order to assess the antioxidant properties of the compounds occurring in the Caigua extracts and in food supplements produced from this plant. Pros and cons, as well as complementary aspects, of HPTLC and HPLC methods for the identification and quantification of biologically active compounds in plant extracts are illustrated and discussed.

Evaluation of excess and adsorption isotherms in chiral stationary phases

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The adsorption equilibria of trans-stilbene oxide (TSO) enantiomers have been studied on Whelk-O1 chiral stationary phases (CSPs) prepared on both core-shell and fully porous particles (FPPs)[1]. Three columns were used in this study: a 100x4.6 mm column packed with 1.8 μm FPPs, a 150x4.6 packed with 2.5 μm FPPs and, finally, a 150x4.6 packed with 2.6 μm superficially porous particles (SPPs).

Firstly, the preferential adsorption of the binary eluent components on the stationary phase (or, more precisely, their excess adsorption isotherms) was evaluated through the so-called minor disturbance method. This method is based on the perturbation of the adsorption equilibria of mobile phase components (in this case hexane and ethanol) through small injections of an excess of one of the two components, when a steady-state equilibrium between mobile and stationary phase is reached. The excess isotherm is then obtained by studying the relationship between the retention times of the so-called disturbance peaks and the composition of the mobile phase[2]. Afterwards, the adsorption isotherms of TSO enantiomers have been studied by modeling overloaded peaks measured under different experimental conditions with mobile phases made of pure hexane and 90/10 hexane/ethanol % (v/v). The isotherm parameters were obtained by iteratively solving the chromatographic mass balance equation adjusting the adsorption parameters until optimal matching between calculated and experimental profiles is reached[3].

Different isotherm models have been used to determine the best adsorption isotherm for TSO on the three columns studied. At 90/10 hexane/ethanol % (v/v) the best adsorption model was found to be the bi-Langmuir one (accounting for two different adsorption sites on the adsorption surface) for all the columns. On the contrary, at 100% hexane the heterogeneous Toth model (predicting a continuous adsorption energy probability density function) best describes the adsorption.

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UHPLC- HRMS Study of Glucosinolates in Cauliflower Waste Products

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Brassicaceae are considered a functional food due to them high content of bioactive compounds, mainly glucosinolates. Several studies have stressed the capacity of Brassica species to prevent some type of cancers, activity connected to the high concentration of glucosinolates in those vegetables. In fact, Glucosinolates are hydrolyzed by endogenous thioglucosidases, called myrosinases, to produce a wide range of degradation products (isothiocyanates, nitriles, epithiocyanates, oxazolidine- 2-thiones, and thiocyanates) with diverse biological activities[1,2].

Frequent consumption of high-GLS-content vegetables is associated with a lowered risk of cancer and cardiovascular disease.

During the processing of these vegetables, an amount of byproducts is produced. Regarding the byproduct proportion, leaves constitute about 50% of the total; the rest is mainly stem. These residues are responsible for important environmental problems in the industries[3] and diminishing their environmental impact has been the subject of an increasing concern in recent years.

However, just a few studies have been done on the waist products of cauliflower's. Nevertheless, the waste still represents a good source of nutraceuticals that can further be subjected to isolation processes.

The aim of this study is to asses different kind of glucosinolates extraction methods, comparing the 80°C extraction against the RT one. Subsequently the purpose is to set up the best chromatographic/mass spectrometric condition in order to characterize the whole glucosinolates composition of cauliflower by-products.

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Air assisted dispersive liquid-liquid microextraction with solidification of the floating organic droplets (AA-DLLME-SFO) and UHPLC-PDA determination of five antibiotics in human plasma of hospital acquired pneumonia patients

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The growing drug resistance of *Gram-positive* (e.g. *Staphylococcus Aureus*) and *Gram-negative* (e.g. *P. Aeruginosa*) bacteria has become, during the last decade, an issue of concern for clinicians. So far, the therapeutic drug monitoring (TDM) of antimicrobial agents could be a useful tool against drug-drug interactions and might increase the therapeutical efficacy in patients suffering from nosocomial infection. Assadi *et al.* in 2006 developed a novel microextraction techinque called dispersive liquid-liquid microextraction[1]. In this technique, a cloudy solution of fine organic droplets is formed when a mixture of extraction solvent (immiscible with water) and a dispenser solvent (miscible with both water and the extratant solvent) is rapidly injected into an aqueous solution sample containing the analytes. In DLLME, large surface contact between fine droplets and the analytes, speeds up the processes regarding mass-transfer of analytes from aqueous phase to organic phase which greatly enhances extraction efficiency[2]. Nevertheless, the extraction solvent used in DLLME is generally highly toxic (chlorobenzene, chloroform or carbon disulfide), not environmentally friendly and incompatible with reverse phase HPLC. Recently, in order to solve this problem, a new microextraction technique has been developed by using extracting solvents which have lighter density than water integrated with the solidification of the organic floating droplet (DLLME-SFO). DLLME-SFO is widely applied for environmental water samples but rarely it is used for the analysis of drugs in complex matrixes such as biological fluids. In the present work a fast, sensitive, precise and accurate method for the determination of MER, MET, CIP, LIN and PIP in human plasma by UHPLC-PDA using a combination of protein precipitation (PP) and air assisted dispersive liquid-liquid microextraction with solidification of the floating organic droplet (AA-DLLME-SFO) has been developed. Various extraction parameters including selection of extractant, amount of extractant, ionic strength, pH and extraction cycles, were tested and optimized. Chromatography was carried out using a Poroshell 120 SB C_{18} (50 x 2.1 mm I.D. 2.6 μm particle size) column and a gradient mobile phase consisting of ammonium acetate buffer (10 mM, pH 4.0) (eluent A); and a mixture of acetonitrile-methanol in a ratio (80/20) (eluent B). Uliflloxacin was used as internal standard. The method demonstrated good linearity with correlation coefficients, $r^2 > 0.9995$ for the drugs, as well as high precision (RSD% ≤ 9.87%), accuracy ranged from -8.14% to +8.98. During the validation, the concentrations of the analytes were found to be stable after 3 freeze-thaw cycles and for at least 24 h after extraction. Subsequently, this method was used to quantify the drugs in patients with HAP in order to establish if the dosage regimen given was sufficient to eradicate the infection at the target site.

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Quality by Design framework in the development of a capillary electrophoresis method for the enantioseparation and impurity determination of cinacalcet hydrochloride

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Cinacalcet hydrochloride (CIN) is the first agent of a new class of calcimimetic compounds, which act by decreasing the secretion of the parathyroid hormone by activating calcium receptors. CIN is the only approved drug by the US FDA in 2004 acting as regulator of ionized calcium and is marketed as single *R*-enantiomer, which is about 75 times more potent than its corresponding *S*-enantiomer. CIN is useful in the treatment of chronic kidney disease where hyperparathyroidism is a common complication of chronic pathologies[1].

In this study, a capillary electrophoresis (CE) method for the simultaneous determination of the enantiomeric purity and of impurities of CIN has been developed following the Quality by Design principles[2]. Comparing to HPLC, CE utilizes the wide spectrum of chiral selectors as buffer additives for chiral separation without the need for expensive columns. Moreover, CE mostly provides shorter analysis time and better resolution and offers the advantage of altering the enantiomer migration order based on the change of the type of chiral selector. The most commonly used chiral selectors are the cyclodextrins (CDs), due to their wide variety of cavity size, side chain, degree of substitution and charge.

In the scouting phase of the development, preliminary experiments were carried out to select the separation operative mode and to identify a suitable chiral selector in order to reach an adequate selectivity. Among the tested CDs, (2-carboxyethyl)-β-CD and (2-hydroxypropyl)-γ-CD (HPγCD) showed chiral resolving capabilities at lower concentration values.

The best results were obtained by using solvent modified capillary zone electrophoresis with the addition of HPγCD. Voltage, buffer pH, concentration of methanol as organic modifier and concentration of HPγCD were investigated as critical process parameters. Critical quality attributes were represented by the critical resolution value between the enantiomers and analysis time. A Box-Behnken Design for four factors allowed the contour plots to be drawn and the design space to be identified with a desired probability level $\pi \geq 90\%$.

The working conditions, with the limits of the design space, corresponded to the following: capillary length, 48.5 cm; temperature, 18 °C; voltage, 25 kV (23-28 kV); background electrolyte, 150 mM phosphate buffer pH 2.8 (2.7-3.0), 3.1 mM (2.2-4.0 mM) HPγCD; 2% (0.0-7.0 v/v%) methanol. Applying the selected conditions, the full separation of analytes was obtained in about 10 min. The method was validated and finally applied to a real sample of CIN tablets.

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Quality by Design approach in the development of an UHPLC method for the analysis of selected polyphenols in *Diospyros Kaki*

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The development of an UHPLC method for the analysis of selected polyphenols in Diospyros kaki, using a triple quadrupole mass spectrometer as detector, is presented. The optimization of the method was performed through the principles of Quality by Design, consisting in the implementation of tools like risk assessment and multivariate strategies.

During the method scouting phase, different types of chromatographic columns were tested, and on the basis of the obtained selectivity an Acquity BEH UHPLC C18 was selected. The Critical Quality Attributes (CQA) were the critical resolutions of some isobaric compounds and analysis time. The Critical Process Parameters (CPP) were selected using the Ishikawa's diagram and were related to the characteristics of both the mobile phase and the column: flow, temperature, initial mobile phase composition, formic acid percentage in eluents, type of organic eluent and gradient. The effects of the CPPs on the CQAs were evaluated by a multivariate strategy, at first using a screening phase and then Response Surface Methodology (RSM). In the screening phase the knowledge space was investigated by an asymmetric screening matrix. Graphic analysis of the effects made it possible to set the type of organic solvent (methanol) and the optimal value for the flow, and in addition to select new experimental domains for the other involved CPPs. In the RSM the postulated quadratic models were calculated by means of Doehlert Design and were graphically represented by contour plots. The search for the global optimum zone was performed by the sweet spot plots, which were analysed in order to identify the zones where the multidimensional combinations of the CPP values allowed the desired values for all the CQAs to be obtained (resolution values ≥1, analysis time ≤20 min). Hence, the Design Space was identified as the multidimensional combination of the CPPs that satisfied the requirements for the CQAs, with a probability $\pi \geq 90\%$.

The method was validated and applied to real samples obtained by the extraction of Diospyros kaki flesh from two different cultivars (*Rojo Brillante* and *Kaki Tipo*) and processed in different ways (fresh fruit without any treatment, treated with ethylene, treated with CO_2). The results showed the remarkable concentration of some compounds (i.e. gallic acid, (+)-catechin, caffeic acid, *p*-coumaric acid, phloridzin), in agreement with literature data. Furthermore, compounds that have not been previously reported were found, including salycilic acid, luteolin, phloretin as well as ellagic acid, that was quantified in high concentration and the presence of which in persimmon was only reported occasionally in the peel of this fruit.

A rapid detection method of 2-dodecylcyclobutanone in irradiated exotic fruit by gas-chromatography / mass-spectrometry (GC-MS) coupled with solid phase microextraction (SPME) technique

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Exotic fruit production represents more than 40% of world fruit production in the world. Exporting exotic fruit worldwide has been made a reality by treating it with ionizing radiation, at the commercial dose levels in the range from 0.5 to 1.5 kGy. The food processing is mainly aimed at reducing spoilage, enhancing hygienic quality and at extending shelf life of treated products with negligible effects on the nutritional profile[1]. According to European legislation[2] any irradiated food, or food containing an irradiated ingredient within the EU must carry the word "irradiated" in a prominent position either as part of the main label or next to the ingredient that has been irradiated. It may also (optionally) show the international icon for irradiated food called the "Radura" symbol. There are several standardized techniques for the detection of the radiation treatment on food based on physical, chemical and biological changes. Among confirmatory methods the European standard EN 1785:2003[3] is able to detect irradiated fatty foods and it is based on the determination of 2-alkylcyclobutanones (2-ACBs), a unique radiolytic compounds formed after the irradiation of lipids components. This method, validated for a limited number of matrices, namely raw chicken, pork, liquid whole egg, salmon and Camembert, is based on the Soxlhlet extraction with hexane of the food lipid fraction, followed by the purification of the lipid phase on a deactivated Florisil column and the detection of 2-ACBs by GC-MS. The major limitations of the EN 1785 method are the Soxhlet extraction step, requiring the use of a large amount of solvent and a long extraction time (about 6 h), and the Florisil column clean-up stage showing a poor capacity to retain fat[4]. In this work, it was set up and validated an alternative method to identify the 2-dodecylcyclobutanones (2-DCB), resulting from the radiolysis of the palmitic acid, extracted by head-space solid-phase micro-extraction (HS-SPME) and detected by GC-MS. Although the HS-SPME-GC/MS technique has been applied on food of animal origin[5] a no study on plant origin food has been reported. Therefore a practical method to detect irradiated exotic fruit, analyzing mango and papaya seeds and avocado pulp, was proposed. Above specific components of food (seeds or pulp) were selected on the basis of their palmitic acid content. Both 2-DCB extraction procedure with a PDMS fiber and quantification of DCB relied on a matrix-matched calibration curve, using an internal standard (IS), was performed. The quantification of DCB and IS by GC/MS analysis was carried out in the SIM mode checking the presence of quantifier ions 98 m/z and 112 m/z in the ratio of 4-4.5:1. Finally main validation parameters such as specificity, LOD, LOQ, precision, intermediate precision and signal stability over a commercial shelf life, were determined. In conclusion an alternative method to current standard EN 1785[3] was successfully developed and applied to detect irradiate exotic fruit. Furthermore the proposed sensitive, rapid and solvent free technique could be extensively used for routine analysis in official controls required by European Commission.

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Protein analysis by two-dimensional LC using size-exclusion and reverse phase chromatography with column switching

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Over the years, two-dimensional Liquid Chromatography (2D-LC) has played a key role in the field of proteomics for the analysis and isolation of proteins from complex matrices, before their characterization by Mass Spectrometry[1,2]. In 2D-LC method development, a large number of experimental variables have to be extensively studied to take full advantage of the claimed features of the chromatographic separations[3]. In the last decade, significant improvements in LC column technology and instrumentation have been made and the introduction of superficially porous particles opened new possibilities in terms of speed and resolution.

Here an automated method combining size exclusion with reverse phase chromatography and UV detection is proposed for protein separations in food samples. For the first dimension separation, a size-exclusion column, packed with 3 μm ultra-pure silica particles densely bonded with a proprietary hydrophilic surface chemistry, was used, ensuring high efficiency and resolution. A reversed phase widepore column was used for the second dimension separation, based on core-shell particle technology that provides striking increases in peak capacity and resolution at lower backpressures. Different collection trap systems were placed on a 10-port switching valve and tested to focus the proteins coming from the first dimension column, before their selective elution in the second dimension column. A protein standard mixture of bovine serum albumin, β-lactoglobulin and glucose oxidase was used to optimize the gradient chromatographic separation conditions, after evaluating their retention behavior in each single column. Finally, the optimized 2D-LC method was applied to the protein analysis in eggs, infant milk powder and soy flour samples.

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The Extractor Naviglio® in Food Productions

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For over a decade the Naviglio Extractor is a good alternative to solid-liquid extraction techniques such as maceration and percolation, as has been amply demonstrated that it is able to provide the same quality (if not more) of the extracts obtained with the traditional extraction techniques, also provides additional benefits such as significant reduction in the time of the extraction process (Ten days of maceration extraction equals one hour of extraction by the Naviglio extractor equal conditions) and a more efficient extraction. The principle on which it bases its functioning (Naviglio's Principle) is studied in graduate courses in Herbal Techniques of several Italian universities. Currently, the Naviglio extractor is widely used in many fields of research and production (herbal, nutritional supplements, homeopatics, cosmetics, beverages). In the food sector, in particular, the Naviglio Extractor has been shown to be a viable alternative to macerate for: production of lemon liquor (limoncello) and similar liquors; and production of bitter elixir of juniper; rapid aging of wines, brandies and liqueurs; extraction of lycopene from tomato processing waste. Finally, have recently been found more unconventional applications of the Naviglio extractor as the rapid hydration of dried vegetables and their simultaneous aromatization, cleaning washers for the production of cork stoppers, cleaning the rubber polymer, the tanning of leather. In Figure 1 laboratory prototype was showed.

NAVIGLIO'S PRINCIPLE: The generation of a negative pressure gradient, using a suitable solvent, between the outside and inside of a solid matrix containing the material to be extracted, followed by a sudden reinstatement of the initial balanced conditions, leads to the forced extraction of those compounds that are not chemically linked to the main structure making up the solid.

FIGURE 1. Laboratory prototype of Esxtractor Naviglio (1998).

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First profiling of flavonoids in Tarocco "Lempso" (*Citrus Sinensis* L. Osbeck) clone variety and its antioxidant potential by DPPH-UHPLC-PDA-IT-TOF

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Clonal selection and hybridization are valid strategies to obtain fruits with enhanced sensorial and nutraceutical properties[1,2]. Within *Citrus sinensis* varieties, Tarocco clone "Lempso" is a typical product of Calabria region (Italy) characterized from a red pulp. This is the first report concerning its accurate profiling.

To characterize in detail the flavonoid composition of Lempso clone and to compare its antioxidant potential with other Citrus varieties by a fast screening method, extracts were subjected to solid phase extraction and the quali/quantitative profile was elucidated through ultra high performance liquid chromatography (UHPLC) coupled to photodiode array (PDA) and ion trap-time of flight (IT-TOF) mass spectrometry detection, and compared to both Cleopatra mandarin (*Citrus reticulata*) and blood orange (*Citrus sinensis* (L.) Osbeck) Sanguinello varieties. The antioxidant activity was assessed by pre-column DPPH reaction coupled to UHPLC-PDA[3].

Lempso is characterized by flavonoids and anthocyanins. Flavanones content (Hesperidin: 57.19 ± 0.49, Vicenin-2: 4.59 ± 0.03, Narirutin: 5.78 ± 0.13 mg/100 mL) was considerably higher than Cleopatra and Sanguinello varieties. The developed DPPH-UHPLC-PDA method provides information regarding the single contributions to antioxidant activity, highlighting how Ferulic acid, Quercetin and Cyanidin derivatives possess considerable radical scavenging activity ($> 50\%$)[4,5]. The total antioxidant activity was also evaluated and compared with positive controls, showing higher scavenging activity than Cleopatra and Sanguinello (IC50: 333.76 ± 10.81 µg/mL vs 452.62 ± 10.81 and 568.39 ± 26.98 µg/mL, respectively).

These data evidence the nutraceutical potential of Lempso variety, which could be an ingredient for functional beverages.

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High Temperature Liquid Chromatography for Lipid Analysis.

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Lipidomics is an emerging field where new analytical techniques are playing an important role for instance in the biomedical research having a wide range of applications in drug and biomarker development [1]. Since the lipids have a large chemical diversity the analysis is characterized by a variety of analytical challenges, above all the solubility issue and the structure similarity within the lipid classes [2]. Porous graphitic carbon (PGC) is a stationary phase with unique chromatographic behavior showing strong retention of both non-polar and polar compounds, the first due to the contribution of the dispersive interactions and the latter due to the interaction with the planar polarizable graphitic surface. The PGC is a suitable stationary for high temperature liquid chromatography (HTLC) since it is resistant to extreme conditions in pH and temperature [3-4]. The retention behavior on reversed phase stationary phases can be predicted using a partial least square modeling (PLS) of structural features combined with chromatographic parameters [5]. A similar approach was used for modelling of retention factors on PGC using chromatographic conditions (temperature, toluene and additive concentration), the structural elements of the compounds (e.g. molecular weight, total carbons and sp2-hybridized carbon), including square and interaction effects. The multivariate regression shows a coefficient of determination (R2) 0.961 for the reference values and 0.948 for the predicted values. The model has been built using the evaporative light scattering detector (ELSD) with chromatographic conditions suitable for mass spectrometry detection. This approach helps to understand the chromatographic behavior exploiting the temperature as a "third chromatographic dimension". Based on the results, a methodology for the analysis of phospholipids based on gradient elution and tandem mass spectrometric detection is suggested

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New modified coated carbon fiber as solid phase for microextraction and in-situ derivatization of phytohormones in real samples.

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Phytohormones play a vital role in plant growth and metabolic processes[1]. As a minor component of the metabolome, phytohormones are of particular significance due to their role in the regulation of germination, growth, reproduction, and the protective responses of plants against stress. In fact, they control the balanced response of plants to adverse environmental conditions or biological threats. Thus, the simultaneous quantitative profiling of different classes of phytohormones provides a useful basis for defining additive, synergistic or antagonistic hormonal activities in biofertilizers or natural compounds. Phytohormones are typically present at trace levels in biological matrices, and the coexistence of a wide variety of interferents make their analysis rather challenging[2]. In this work, an integrated sample pre-treatment and derivatization method based on a new coated carbon fiber microextraction that allows a rapid extraction, separation, and highly sensitive phytohormone detection has been realized and characterized. Carbon fibers, chosen as substrate for their excellent high thermal and chemical stabilities, high porosity, and low cost, were chemically modified to obtain new surface characteristics in terms of polarity and adsorbing capacities. Three phytohormones (jasmonic acid, indole-3-acetic acid and abscisic acid) were simultaneously extracted, in-situ derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide and directly subjected to analysis by gas chromatography–mass spectrometry. The developed method allowed in few step and short time low detection limits ($0.62 – 3.72$ ng mL^{-1}), good linearity (in the range $3.0 – 5000$ ng mL^{-1}) and recoveries (81.6 to 105.8%). Furthermore, enrichment response factors (calculated as the ratio of peak areas obtained by SPME thermal desorption and direct injection) up to 3200, high derivatization rates ($\geq 90\%$) and good reproducibility (RSD $\leq 20\%$ at 50 ng mL-1) were obtained. The developed method was applied for the evaluation of phytohormone concentration in blended tomato samples.

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A high throughput mass spectrometry screening analysis based on two-dimensional carbon microfiber fractionation system

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Compounds identification and quantification in complex samples are still a great challenge[1,2], because real matrices are generally constituted by an impressive number of substances, which shows great differences in physical and chemical properties, requiring then complex pretreatment, separation and analysis procedures[3]. In particular, polarity plays a great role in this perspective, making their simultaneous detection and quantification very demanding. Another critical issue that should be addressed is the great difference in the substance content (from mg g^{-1} to ng g^{-1}), that can lead to mask trace compounds or to saturate the limited capacity of the separation columns: most of them remain unknown or not revealed[4]. A quite interesting case-of-study in this field is the analysis of medicinal plant extracts. Their composition can provide very important information in metabolomics studies, in the comprehension of the synergistic effects of major and minor components for the plant resources exploitation and for the knowledge of therapeutic mechanisms[1,4]. The high complexity of these extracts, containing both polar, medium polar and non-polar substances lead to very complex, tedious and time-consuming analytical procedures for their analyses. One possible solution to overtaking all these drawbacks can rely on a new analytical approach that gathers a pretreatment that split molecules with different polarities in distinct fractions with direct MS analyses[5]. Our research has been focused then toward the realization of a system that could allow this separation in a fast, reliable and inexpensive way. A novel high-throughput, solvent saving and versatile integrated two-dimensional microscale carbon fiber/active carbon fiber system (2DμCFs), that allows a simply and rapid separation of compounds in low-polar, medium-polar and high-polar fractions, has been coupled with ambient ionization-mass spectrometry (ESI-Q-TOF-MS and ESI-QqQ-MS) for screening and quantitative analyses of real samples. 2DμCFs led to a substantial interference reduction and minimization of ionization suppression effects, thus increasing the sensitivity and the screening capabilities of the subsequent MS analysis. The method has been applied to the analysis of *Schisandra Chinensis* extracts, obtaining with a single injection, a simultaneous determination of 33 compounds presenting different polarities, such as organic acids, lignans, and flavonoids in less than 7 min, at low pressures and using small solvent amounts. The method was also validated using 10 model compounds, giving limit of detections (LODs) ranging from 0.3 to 30 ng mL^{-1}, satisfactory recoveries (from 75.8 to 93.2%) and reproducibilities (relative standard deviations, RSDs, from1.40 to 8.06%).

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Preparation of Nanoemulsions From Peel Extracts Of Citrus Fruit Obtained By Rapid Solid Liquid Dynamic Extraction (RSLDE)

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Citrus fruits are a source of several characteristic compounds which are responsible for their odour and biological proprieties.

To be more detailed, extracts of citrus fruit peels may show antimicrobial, anti-inflammatory and haemostatic activities which are due to a number of inorganic compounds (e.g., phosphorus, calcium, copper), essential oils (e.g., terpenes), fibers, vitamins and fats.

So much so that they have several applications in the perfume and cosmetics industry as well as in the food, beverage and confectionary industries as flavouring agents for liqueurs, teas, candies and soft drinks.

However, the final properties of the citrus fruit peels crude extracts strongly depend on the details of the extraction process.

Cold pressing and hydrodistillation are presently the most used techniques employed in the production of fruit peels extracts which, however, contain mainly essential oils.

Here we present results on the composition of citrus fruit peels extracts obtained by a very performing and inexpensive extraction technology based on Rapid Solid Liquid Dynamic Extraction (RSLDE) implemented via an automated apparatus (Naviglio Extractor).

Interestingly, both volatile and non-volatile components were detected in crude extracts characterized mainly via GC/MS analysis.

Then, very stable ultra-small oil in water emulsions (with oil droplets less than 20 nm in diameter) have been produced by combining RSLDE with emulsification of extracts by a high-pressure homogenizer. Additionally, the oil is under investigations in terms of bioactivity.

This is an intriguing aspect of our investigations because of the potentialities of these oils in the field of biotechnology due to their therapeutic activities. Indeed, attempts to prepare emulsions from similar commercial oils obtained with usual extraction methods resulted unsatisfactory.

MSPD Extraction And UHPLC-ESI-MS/MS Characterization Of Selected Phenolic Acids And Flavonoids

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Matrix solid phase dispersion (MSPD) is an extraction method that allows in few steps the extraction/isolation of a widespread range of molecules, with limited sample and lower solvent quantities consumption (1). In this technique, the investigated sample is blended with a chemically derivatizated solid support; the resulted mixture is packed in a cartridge next subjected to a washing and an elution steps. Thanks to hydrophobic and hydrophilic interactions that occur, in these steps the interference removal and the extraction/isolation of the target analytes are obtained. This technique can be applied to complex matrices and to any target analyte[2].

In the whole of the different fields in which MSPD can be applied, we focused our attention to plant polyphenolic compounds (PCs). These molecules are secondary metabolites with different structures and functions. Among them, we selected phenolic acids and flavonoids classes, which have already displayed interesting biological properties in human health[3].

The optimization of the MPSD different steps and conditions was performed on different silica based sorbents (C2, C8, C18E-capped, C18T-uncapped, phenyl) and 16 selected analytes with different polarity and representatives of the subclasses of which they belong. The MSPD procedure was followed by an UHPLC-ESI-MS/MS analysis.

The most suitable MSPD protocol turned out to be the following: C18-T as bonded phase, 1 mL of 90:10 water/methanol solution for the washing step and an elution step with 500μL of methanol. After the preliminary results, the method was successfully implemented with a 35analytes of the same selected classes.

The method was also applied to *Ocimum basilicum,* the common basil worldwide used in food flavoring. The obtained results present good analytical performances in terms of repeatability, accuracy, linearity and matrix effect.

The optimized MSPD-LC-MS/MS method can be adapted to similar matrices and could provide a valid basis for additional or similar investigations of other plant bioactive compounds.

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Investigation on the Role of Carbohydrate Moiety in the Catalytic Activity of a Laccase from Trametes Versicolor

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Laccases (EC 1.10.3.2) are polyphenol oxidases able to catalyze the oxidation of various organic and inorganic compounds with the concomitant four-electron reduction of molecular oxygen to water. Typical substrates are particularly those with electron-donating groups such as phenols ($-OH$) and anilines ($-NH_2$). An important feature of this kind of biomolecule is the high level of glycosylation, with covalently linked carbohydrate moieties ranging from 10-50% of the total weight, which may contribute to the high stability of the enzyme[1]. The oxidation of carbohydrate residues by periodate is a common strategy for the site-specific modification/immobilization of antibodies in order to apply them in various bioanalytical methods. According to this approach, diols located in the carbohydrate chains are cleaved by periodate to form reactive aldehyde groups or hydrazide residues[2]. In the present study, the possibility of improving the immobilization yields of a Trametes versicolor laccase on chitosan *via* the same oxidation protocol is proposed, in order to explore the role of carbohydrate moiety through the modification of monosaccharides covalently attached to the enzyme. As a matter of fact, the immobilization of enzymes has been usually accomplished by several procedures, most of which include the covalent attachment of the enzymes to various matrices through a cross linking agent[3]. In this work laccase was submitted to undergo periodate oxidation and, after the elimination of the remaining periodate through a dialysis step, the oxidized glycoprotein is directly incubated with chitosan macro particles. The buffer favourable conditions and the optimal laccase concentration for the immobilization were investigated as well as the aldehyde groups formed after the oxidation were qualitatively and quantitatively determined through 2,4-dinitrophenylhydrazine spectrophotometric assay[4]. The bioreactor was then characterized for kinetic parameters (Michaelis-Menten constant and maximum rate), reuse, storage stability and pH/temperature profiles. The study of the best operative conditions is useful for the application of the biocatalysts in several biotechnological processes. The oxidation procedure has been proved to be feasible for the preparation of stabilized glycoproteins without the introduction of foreign molecules suggesting that the carbohydrate moiety of laccase, involved in the interaction with ammoniac groups of chitosan, doesn't appear to be closely related with the catalytic site of the protein.

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Monitoring peptides released after gastro-intestinal digestion by online comprehensive LC × UHPLC-HRMS: A case study on buffalo milk dairy products

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Dairy products contain many bioactive peptides that are encrypted in the sequence of precursor proteins and become bioaccessible and active after release during gastro-intestinal digestion[1]. The resulting matrix are often very complex, containing hundreds of compounds. Conventional analytical techniques based on monodimensional liquid chromatography methods coupled to mass spectrometry are not capable to handle this challenging samples and thus high peak capacity values are necessary[2]. In this regard, we developed an online comprehensive two-dimensional liquid chromatography platform by two coupling two reversed phase columns operating at different pH values[3]. In the first dimension, a microbore RP column was employed whereas in the second dimension two different short sub-2 μm stationary phases were compared: a fully porous monodisperse C18 column and a core-shell C18 column[4]. The peptides were monitored by UV detection and identified by tandem mass spectrometry (MS/MS). The developed method provided double peak capacity values with respect to monodimensional methods and high orthogonality, together with a major number of identified peptides and a quick visualization of matrix differences by 2D map comparison. The method is highly suitable for peptidomics studies[5].

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Dried Blood Spot analysis for therapeutic drug monitoring: determination of ibuprofen, uric acid and creatinine by UHPLC-MS/MS

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The aim of this work was to develop an innovative UHPLC-MS/MS method for the determination of ibuprofen, creatinine and uric acid in dried blood spots (DBS) collected from preterm infants with Patent Ductus Arteriosus (PDA). PDA represents a hot topic in neonatology, whose pathophysiology, diagnosis and management are still a debated issue. Ductal patency is primarily due to an increased oxygen tension and high prostaglandin-E_2 (PGE_2) levels, which are suppressed by cyclooxygenase inhibitors (e.g. ibuprofen). The choice of treatment is challenging as it depends on a complex risks/benefits analysis due to frequent failures and severe adverse effects of drug (e.g. impaired renal function and intestinal perforation). The present approach can be a potential tool for the non-invasive, rapid, and painless monitoring of ibuprofen treatment and its main pathophysiologic effects on the kidney of preterm infants suffering from PDA. The analytical procedure combines a fast and reliable extraction of the analytes from a single drop of blood and a highly sensitive and selective UHPLC-MS/MS technique. Whole blood (20 µL) was spotted onto a filter paper, dried, and then extracted and diluted with mobile phase prior to analysis. The sampling and extraction steps were optimized by testing two types of sample collection cards, (i.e. Whatman FTA DMPK-B and Whatman-903), and various water/methanol mixtures. The analytical figures of merit, i.e. linearity, detection and quantification limits, intra- and inter-day precision and recovery, are finally discussed.

Individual and Cyclic Estrogenic Profile in Woman: Structure and Variability of the Data

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Women of childbearing are the ones who still have the period, thus the amount of estrogens, produced by the ovaries, results cyclic with wide fluctuations throughout the menstrual cycle.

To date, the variations of the principal estrogens (estradiol, estrone and estriol) were only evaluated, consequently the aim of the study was to examine in depth the cyclic fluctuations of some metabolites.

A Gas Chromatography coupled to Mass Spectrometry (GC-MS) method capable of detecting estradiol, estrone, estriol and their metabolites in urine was developed, based on an experimental design, and fully validated.

The following validation parameters were investigated according to ISO 17025 requirements: linearity range, selectivity, specificity, limit of detection (LOD), limit of quantitation (LOQ), trueness, intra- and inter-assay precision, repeatability, matrix effect, extraction recovery and carry-over.

A wide panel of estrogens, consisting of 17α-estradiol, 17β-estradiol, estrone, estriol, 4-methoxyestrone, 2-methoxyestrone, 16α-hydroxyestrone, 4-hydroxyestrone, 2-hydroxyestrone, 4-methoxyestradiol, 2-methoxyestradiol, 4-hydroxyestradiol, 2-hydroxyestradiol, 16-epiestriol and 17-epiestriol, was monitored in some young healthy women throughout a complete menstrual cycle.

The resulting data were simultaneously evaluated through the application of the Parallel Factory Analysis (PARAFAC) method, in order to explore the variability of each analyte and each class of estrogens.

The estrogens, furthermore, play a significant role in the development of ovarian and breast tumors. In fact, these carcinomas are closely related to the alteration of the estrogenic production.

Urine samples, collected from healthy post-menopausal women and volunteers affected by breast cancer, were analysed.

Post-menopausal women were chosen because of the higher incidence of these tumors and the greater stability of the urinary estrogenic concentration compared to young women.

The evaluation of the estrogenic profile was performed and the resulting data were analysed by multivariate statistic techniques. Among the most important techniques, Discriminant Analysis (DA), Partial Least Square – Discriminant Analysis (PLS – DA), Principal Component Analysis (PCA), Cluster Analysis and Soft Independent Models of Class Analogy (SIMCA), were tested in order to discriminate between healthy women and the ones suffering from carcinomas.

Phospholipid profiling of donkey milk by HILIC with electrospray ionization and Fourier-transform high resolution/accuracy mass spectrometry

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Bovine milk represents an important source of essential nutrients which contribute to the growth and development of newborns when breast feeding cannot be provided. Unfortunately, approximately 2–3% of infants younger than one year have reported allergy to cow milk[1]. Many clinical researches have demonstrated that donkey milk can be considered a potential alternative to cow milk intolerant infants due to its very similar composition to human milk[2]. For this reason, several studies have been focused on the proteome[3] and peptidome[4] characterization of donkey milk. As far the lipid fraction is concerned, a number of authors investigated triglyceride (TAG)[5] and fatty acid composition[6] showing that, like human milk, TAG C52 was the most abundant; saturated fatty acids, predominantly palmitic acid, were present mainly in the *sn-2* position of the glycerol backbone[5]. To our knowledge, few data are accessible on the minor polar lipid species of donkey milk fat as phospholipids (PL)[7,8].

The aim of this study was to characterize these minor lipids on a pool of samples of donkey milk collected from Martina Franca breed. Phospholipid classes were separated based on the polarity of their headgroups using hydrophilic interaction liquid chromatography (HILIC) by gradient elution with acetonitrile and water containing formic acid and ammonium acetate as mobile phase. A solid-core 2.6 μm HILIC column was employed at a flow rate of 0.3 mL/min at ambient temperature. Structural characterization of PLs was performed by electrospray ionization with high-resolution/accuracy Fourier-transform Orbitrap mass spectrometry (ESI-FTMS) and tandem MS. For a complete depiction of as many as possible PL molecular species, the analyses were performed in positive, negative and negative with in source fragmentation ion mode. Indeed, in the last modality a higher number of product ions were attained in MS/MS analyses of phosphatidylcholines (PC) and sphingomyelins allowing a more confident characterization. It was found that phosphatidylethanolamines, phosphatidylcholines and sphingomyelins were the most abundant PL, followed by phosphatidylinositols, lysophosphatidylethanolamines, and lyphosphatidylcholines. Besides, the presence of hexosylceramides, gangliosides and N-acetylphosphatidylethanolamines was assesses for the first time in donkey milk.

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Label free shotgun proteomics approach to characterize muscle tissue from farmed and wild european sea bass (Dicentrarchus labrax)

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Recently, aquaculture has become central to meet the increased fish demand; according to the Organization for Economic Cooperation and Development (OECD) and to the Food and Agriculture Organization (FAO), in 2022 the world aquafarming will be increased by a 35% factor, while traditional fishing only by 5%, due to population growth, rising incomes and increasing urbanisation[1]. During farming, fish species are subjected to a plethora of stimuli and environmental constraints such as different diet, feeding regiments, crowding, different water quality (temperature, salinity), with respect to wild fish species. The nutritional characterization of aquaculture products represents an important step toward a quality certification process of seafood that informs the consumer about the origin, handling practices, and processing history of the products, thus guaranteeing safety requirements and nutritional properties[2]. Under a nutritional perspective, studies on fatty acid and lipid compositions between wild and aqua-farmed individuals have been playing a major role in the past years leading to the publication of many papers in the literature, which highlight that omega-3 fatty acids were naturally more concentrated in wild fish, whereas their presence in farmed fish was lower in favor of omega-6 and oleic acid, as an evident consequence of food supplementation[3]. In the last years the study of protein content has also become particularly important for the characterization of food products and to assess food quality and safety[4]. Proteomics can be exploited for sea food as well, in order to distinguish fish species, trace their origin and compare the farmed product to the wild one. Differences may thus be expected. In this study, a proteomic profile of farmed and wild sea bass samples was performed, employing a fractionation strategy where peptide samples were first separated by high-pH RP and then by low-pH RP chromatography. The peptides were finally analyzed by a typical shotgun proteomics workflow with high resolution tandem MS and label free quantitative analysis. The chosen fractionation approach was successful, with 70% of the identified peptides detected in a single fraction. This allowed to greatly improve the fish muscle protein characterization and detect some interesting differences between wild fish and farmed sea bass. 69 proteins were overexpressed in farmed fish samples, whereas 182 proteins were underexpressed. Some of these proteins could be related to the breeding conditions and the diet with which fishes were fed, thus providing some interesting results for assessing food quality based on a comprehensive proteomic study.

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A rapid microextraction by packed sorbent - liquid chromatography tandem mass spectrometry method for the determination of dexamethasone disodium phosphate and dexamethasone in aqueous humor of patients with uveitis

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Uveitis, i.e. inflammatory disorders of the uveal tract of the eye, represent a particular burden of disease during adulthood and frequently cause cataract formation. Supplementation with corticosteroids is usually recommended as perioperative prophylaxis in cataract surgery. In particular, in topical ophthalmic products, the dexamethasone disodium phosphate (DEX-SP), a glucocorticoid that can be hydrolyzed to free desamethasone (DEX) by phosphatase, is one of the most prescribed worldwide to treat ocular inflammations. The topical administration of this drug still remains ideally preferable, however its major drawback lies in its poor permeability towards the corneal epithelium (less than 3% of the instilled dose reaches the aqueous humor)[1].

The analysis of dexamethasone and dexamethasone sodium phosphate in aqueous humor requires high sensitivity and selectivity due to the presence of these compounds at low concentration levels. Another drawback lies in the low amount of biological sample that can be collected during surgery. In this context, we developed and validated a new method based on microextraction by packed sorbent (MEPS) coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the determination of DEX and DEX-SP in human aqueous humor with the final goal of evaluating the effectiveness of the glucocorticoid topic administration. In a research program aimed to the development of extraction methods for rapid, sensitive and high-throughput MS-based analysis[2], the use of MEPS was proposed as miniaturized extraction technique, since it permits to combine sample processing, concentration and clean-up into a fully-automated online sampling/injecting device and to process reduced sample volume[3]. A central composite design and the multicriteria method of the desirability functions were applied for optimization of MEPS conditions allowing to find the best conditions for the simultaneous extraction of both the analytes. Detection was performed on a LTQ XL linear ion trap mass spectrometer using electrospray ionization in positive ion multiple reaction monitoring mode. Validation of the assay showed quantitation limits of 0.7 and 0.5 µg/L for DEX-SP and DEX, respectively, a very good precision (RSD ≤ 16%) and recovery rates in the 91 – 119% range[4]. The method also demonstrated rapid analysis time, low volume consumption and good selectivity, thus being a valuable tool for clinical studies to assess the reliability of dexamethasone administration in patients affected by uveitis.

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In silico modeling tools for the overproduction of molecules with a biotechnological interest: experimental validation of model predictions on *Streptomyces ambofaciens*

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Both academic and industrial laboratories currently use constraint-based reconstruction methods to predict optimal genetic modifications aiming at improving the yield of chemical production[1]. *Streptomyces ambofaciens*, a prolific producer of bioactive compounds has been studied with different modeling tools[2]. The interest is linked to its ability to produce a wide range of secondary metabolites such as spiramycin, kinamycin, antimycin and stambomycins, and novel polyketides with antibacterial and antiproliferative activities[3]. In this study, the metabolic pattern of *Streptomyces ambofaciens* has been globally explored: a set of candidate overexpression gene targets supposed to lead to spiramycin overproduction have been evidenced through metabolic modeling. Model predictions were experimentally validated by genetic manipulation of the ethylmalonyl-CoA metabolic node, providing evidence that spiramycin productivity may be increased by enhancing the carbon flow through this pathway. The goal was achieved by over-expressing the *ccr* paralog *srm4* in an *ad hoc* engineered plasmid. The first metabolic reconstruction of *S. ambofaciens* and the successful experimental validation of model predictions have been described and the validity and the importance of *in silico* modeling tools for the overproduction of molecules with a biotechnological interest demonstrated. As a result, the proposed metabolic reconstruction represents a solid platform for the future exploitation of *S. ambofaciens* biotechnological potential[4].
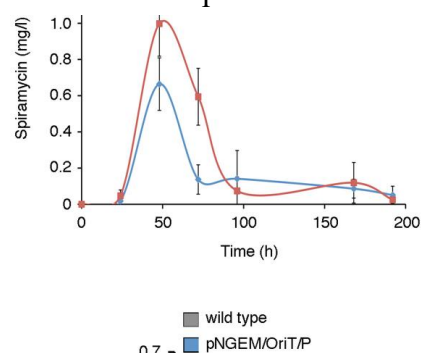
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A promising approach to early detect Sickle Cell Anemia

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Sickle Cell Anemia (SCA) is the most common inherited hemoglobin disorder, associated with recurrent painful episodes, ongoing hemolytic anemia and progressive multi-organ damage. It is so defined because it imparts to the red blood cells (RBCs) a sickle shape. SCA is caused by the presence of an abnormal hemoglobin where the glutammic acid is replaced by the valine in the β chain, leading to the formation of hemoglobin S (HbS)[1].

The clinical overview is very heterogeneous and it is characterized by chronic hemolysis and vaso-occlusion due to the polymerization of HbS into the RBCs[2].

A multidisciplinary approach to diagnosis of sickle cell disease is usually required and often it is a problem, especially in countries where the resources are scarce or absent. To develop quick and effective method for a preliminary detection of hemolytic anemia has been become very important.

Based on previous results achieved for the β-Thalassemia syndromes, the coupling of Thermogravimetric Analysis (TGA) and chemometrics was investigated to develop a model of prediction for Sickle Cell Anemia[3].

Whole blood samples from healthy subjects and falcemic subjects have been investigated by Thermogravimetric Analysis and the resulting TG and DTG curves were considered for the chemometric analysis to identify a characteristic thermal behavior among the two populations according to a different amount of water content and corpuscular fraction.

The same samples has been characterized to confirm the presence or absence of hemolytic anemia by evaluating the characteristic hematological features: Hemoglobin content (HGB), Hematocrit (HCT), Red Blood Cells (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MHC) and Red-cell Distribution Width (RDW).

The preliminary outcomes are promising and allows to consider coupled TGA/Chemometrics an alternative, cheap and fast diagnostic method for a early detection of Sickle Celle Anemia.

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In-depth Proteomic Study of Plasma Proteins During Sport Activity

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In recent years, more and more people have joined the world of running and this increase is even more pronounced for marathon and half marathon. Physical activity improves overall health and counteracts metabolic pathologies. It is also well-known that exhaustive exercise is associated with free radical (FR) production and consumes the available antioxidants, especially during periods of intensive training. Depending on the nature, degree and duration of the physical activity, the different cell types of the body suffer varying levels of oxidative stress, which may threaten the athlete's well-being. The purpose of our study was to investigate plasma protein profiles of athletes before and after a half marathon. The immunodepletion of abundant proteins followed by untargeted mass spectrometry analysis allowed us to compare pre- and post-exercise blood samples in order to provide both qualitative and quantitative information. Interestingly, proteomic data showed the activation of both inflammatory response and detoxification process. In addition, the involvement of pathways associated to immune response, lipid transport and coagulation, was elicited. In addition, for the first time we have analyzed the proteomic profile changes in runners after half-marathon activity in order to survey the related systemic adjustments.

Plasma Proteome Profiles of Stable CAD Patients Stratified According to Total Apo CIII levels

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Apolipoprotein C-III was recently acknowledged as a prognostic marker for cardiovascular risk. High levels of Apo-CIII strongly correlate with hypertriglyceridemia and are associated with atherosclerosis and coronary artery disease (CAD).

Aim of the present research was to study the plasma proteome profiles of stable CAD patients characterized by different levels of total Apo-CIII. Two subgroups of CAD patients with divergent concentrations (under and upper the median concentration of the population distribution=10 mg/dL) of total circulating Apo C-III were examined using a shotgun proteomic approach. Through the use of a micro-LC SWATH-MS analysis we were able to quantify 180 plasma proteins: the data were then analyzed by bioinformatic tools and multivariate statistics. The fold change analysis and the Partial Least Square Discriminant Analysis showed a clear separation of the two groups. The dynamic processes that involve Apo C-III concentration in blood and its relation with all other plasma proteins were considered. The concentrations of Apo C-III, Apo E, retinol-binding protein 4 and alpha 1-antitrypsin were also validated using ELISA assays.

In this pilot study, the different expression of plasma proteins through the entire range of concentrations of Apo C-III was defined, suggesting possible new players involved in the APO C-III-associated process of arterial damage.

Cholesterol Content of Two Eggs a Day in Human Supply Noes Not Harm the Health of Healthy Individuals

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Chicken eggs are highly favored foods for their nutritional and functional properties due to the high content of proteins, lipids and micro nutrients e.g. vitamins and minerals. In the past years has been reported that the cholesterol contained in the egg yolk was an high contribution to the cholesterol recommended daily intake and consequently the nutritionists recommended a minor weekly consumption of eggs. In this study, the quantitative determination of cholesterol in chicken egg yolk was investigated. There are some conflicting views and results in relation to the analytical methods used for the cholesterol determination. An efficient, fast and reproducible method for cholesterol determination is then vital for the control of cholesterol uptake. This paper proposes a simple method for the rapid quantification of cholesterol in chicken eggs yolk by gas chromatography that could be used as a routine method for the rapid and sensitive determination of cholesterol.

Cholesterol content in a sample of 100 eggs purchased on the local market, ranging in 55-80 g interval of weight was analyzed. The results indicate that the cholesterol content is in the range of 140 - 210 mg (average value 175 mg/egg) instead of 250 mg/egg as reported in the literature. The difference of cholesterol content before and after transesterification, indicates that the cholesterol content as sterylester in the egg fatty part is negligible. The analytical procedure used, based on gas chromatography (Figure 1), allowed us to quantify the non polar fraction of the egg yolk fat, which is about the 75% of the total yolk fat weight, while the cholesterol content of the yolk is not related to the weight of the yolk itself and to the weight of the entire fatty part. The obtained results do not agree with the previously reported data allowing us to conclude that the cholesterol content in poultry eggs has been overestimated.
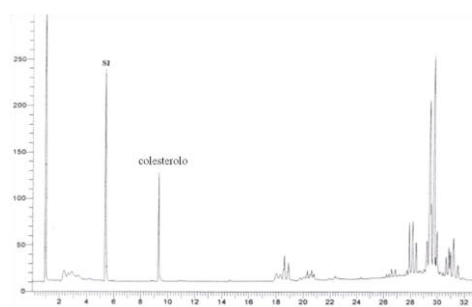
FIGURE 1. Gas chromatogram of free non polar lipid fraction of egg's fat. Cholesterol peak is separated from triglycerides and analyzed by means of internal standard (squalene). Column 65% phenyl methylslicone HT (TG), RTX 65-TG (Restek, Bellefonte, CA, USA); l=30 m; i.d.=0,25 mm; f.t. 0,25 μ.

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LC-ES-MS/MS-Assisted Bile Acids Speciation In Biological Tissues In Permeability Studies Using Franz Cell Model.

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The intestinal adsorption of natural and new semisynthetic bile acid (BA) play a major role in their organ biodistribution and biological activity. BA as detergent-like compounds self-aggregate to form micelles with phospholipids and cholesterol. The critical micellar concentration and the micellar stability constant, pH, ionic strength plays a major role in the equilibrium between BA monomers and micelles and the related partition and diffusion across biological membranes. The monomer passive diffusion is related to its lipophilicity as evaluated by the octanol-water partition coefficient LogP. BA with different physicochemical properties including cholic, chenodeoxycholic, deoxycholic and 6-ethyl chenodeoxycholic acids have been studied for their permeability in biological tissues. It is generally considered that the BA physico-chemical characteristics are the most important factors contributing to their partition, permeation and diffusion. BA, if able to across membranes (LogP>1), diffuse in the layered tissue compartments and their permeability coefficient (Kp) calculated according to Fick's first law of diffusion, based on the steady state flux allow to evaluate the passive process efficiency. Moreover, additional thermodynamic interactions with binding proteins and specific receptor are involved in the net permeation process and therefore BA could be present in the biological fluids and compartment in different species, such as neutral/ionized monomer, simple BA micelles, mixed micelles, monomer-protein complex, monomer-receptor complex, all of these equilibrium species modulated by the pH and ionic strength. In order to define the species involved, an integrated analytical approach has been developed. BA were identified and quantified in the different biological fluids such as bile, simulated duodenum content, tissue sub-fraction by HPLC-ES-MS/MS as previously reported[1]. The method fulfils all the standard criteria and the limits of quantification (LOQ), were < 20 nM for each analyte. Surface tension experiment were performed using maximum bubble pressure method allowing to define the monomer-micelles equilibrium and the CMC. The LogP was measured as a function of the pH, ionic strength and BA concentration. Data of protein binding and specific receptor such as TGR5 (membrane) and FXR (cellular) thermodynamic affinity constants will be used to define the complex multiple equilibria present in the intestinal content/ tissue stirred and unstirred interface. The Franz diffusion tissue system using vertical cell has been used to measure the BA permeability as a function of the pH, initial concentration and ionic strength. The steady state flux (J, μg cm^{-2} h^{-1}) and lag time can be estimated. Data show that a BA with a high LogP such as DCA, despite an efficient partition in the tissue, present a delayed clearance from the tissue due to a strong interaction with the lipid domain of the tissue and additional interaction with FXR receptor. An intermediate LogP such as that of CA allows a more efficient and fast process. Modeling, understanding and characterizing the permeation process of BA with different structure and physicochemical properties through various biological membrane barriers and tissue is essential in order to predict the *in vivo* behavior and to explain their mechanism.

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The Combined Use Of Alkyl Chloroformate And Solid-Phase Microextraction For A Fast And Easy Assay Of Polyamines In Human Urine By Gas Chromatography-Triple Quadrupole Mass Spectrometry

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One of the decisive aspects to fighting cancer in an effective manner is early diagnosis and rapid treatment of this disease. In this respect, the development of new, rapid and cheap tumor diagnostic techniques represents one of the main targets of recent clinical research. Several studies have been focusing on the discovery of cancer biomarkers because their identification can be of crucial importance in early detection programs. Indeed, a high concentration of these molecules or in some cases just their detection can provide information on tumor occurrence, type of cancer, and disease progression at very early stages[1].

Polyamines are aliphatic amines with low molecular weight, which are essential for normal growth and cellular differentiation[2]. The marked increase of the biosynthesis of polyamines has been associated with rapid tumor growth, resulting in the increase of their level in urine and plasma[3]. Due to these findings, it is widely recognized that the polyamines are among the most important cancer biomarkers for early diagnosis and treatment.

The goal of this work is the development of a rapid and simple method for the quantification of free polyamines (i.e., putrescine, cadaverine, spermidine, spermine) and N-monoacetylated polyamines (i.e., N^1-Acetylspermidine, N^8-Acetylspermidine and N^1-Acetylspermine) in human urine. A preliminary derivatization with propyl chloroformate combined with the use of solid phase microextraction (SPME) allowed for an easy and automatable protocol involving minimal sample handling and no consumption of organic solvents. The affinity of the analytes toward five commercial SPME coatings was evaluated in univariate mode and the best result in terms of analyte extraction was achieved using the divinylbenzene/carboxen/polydimethylsiloxane fiber. The variables affecting the performance of SPME analysis were optimized by the multivariate approach of "Experimental design" and, in particular, a central composite design (CCD) was chosen. Analyses were carried out by gas chromatography-triple quadrupole mass spectrometry (GC-QqQ-MS) in selected reaction monitoring (SRM) acquisition mode. The developed method was validated according to the guidelines issued by the Food and Drug Administration (FDA). The satisfactory performances reached in terms of linearity, sensitivity, matrix effect, accuracy and precision make the proposed protocol suitable to be adopted for quantification of these important biomarkers in urine samples.

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A Workflow For ESI-MS High Resolution Lipidomics Data Processing

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Lipids comprise a diverse group of biomolecules organized in height major categories with a considerable range of structural attributes and chemical properties. Great attention has been recently devoted to those compounds since it has been observed that variations in lipid profile may be related to several human diseases. Typically, individual species are identified and quantified with mass spectrometry (MS)-based platforms using sample preparation protocols and synthetic lipid standards if a targeted approach is followed. Alternatively, lipids can also be profiled with untargeted analysis, in which no synthetic lipid standards are used for quantification and lipid abundance is inferred from the arbitrary intensity values usually normalized to each class. In the latter case, the analysis of lipidomics data requires automation with the support of dedicated software. In the last years, several efforts have been shaped with the aim to analyze data from either shotgun experiments such as LipidView[1], LipidXplorer[2], ALEX[3]] or from liquid chromatography (LC)-MS such as LipidSearch[4] and LIMSA[5]. Note however that the only matching of *m/z* values without further data processing and validation can represent a major source of error. Thus, a first data deconvolution is necessary by using algorithms which allow for correct identification and correct isotopic overlap. Of course, a validation of results through the visualization of input data and obtained results is always required. By managing big lipidomics data sets, every proposed and above mentioned package exhibits some advantages and limitations. For instance, ALEX lipid database currently covers about 20,000 species divided in more than 85 lipid classes. Each lipid species in the database is annotated using the chemical formula, the mono-isotopic mass in both the positive and negative ionization mode, the lipid group, the lipid class and other information. More, ALEX database is also available as Microsoft Excel file to help enumerate and calculate formula and chemical mass for each species, so that the user can easily add a lipid class. On the other hand, it does not allow to correct for isotopic overlap, it is specific for high resolution MS and it is quite difficult to manage. By using LIMSA Excel add-in it is possible to find and integrate peaks in a mass spectrum for comparison with an internal list. This tool is also capable of correcting the overlap of isotopic patterns and quantify the lipid species identified by using internal standards. LIMSA has a friendly user interface and can be applied to any type of MS spectrum. LIMSA internal database cab be implemented by adding new lipid classes. A disadvantage of LIMSA is related to the assignment of some lipid species which are based only on *m/z* feature matching, providing an extensive list of "misleading" and improbable identifications. Conversely, ALEX database proposes more appropriate lipid assignments considering fatty acids really occurring in nature. The idea of this work is to integrate ALEX into LIMSA database with the aim to join the advantages of each software creating a new easy lipidomic platform. To do this, ALEX database has been downloaded and used as starting point for building a new written database that can be implemented into LIMSA. Finally, the whole developed workflow is applied to the lipidome analysis of a complex real sample of human plasma.

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Emerging Contaminants In Food: Analysis Of Chocolate By UHPLC Coupled To High-Resolution Mass Spectrometry

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One of the great challenges in food safety is to assess the risks associated with the presence of emerging contaminants, chemical substances not mentioned in the Regulation but representing a potentially risk to human health. Emerging contaminants in food are increasing as a consequence of new industrial processes, agricultural practices, environmental pollution. Foods can be contaminated during the entire life of a product, from the collection of raw materials, during the whole production chain, to storage and consumption. Possible contamination sources are represented by parts of industrial plant or plastic objects in contact with the product also during meal preparation[1]. Since contamination may imply a risk to health, and has a negative impact on food quality, the European Union has taken measures to minimize their occurrence in foodstuffs. Therefore, an important trend of analytical chemistry is to develop fast and efficient procedures for the trace analysis of target and non-target compounds in food. Many researchers centered their attention on the analysis of simulants, while only few papers report results based on real food samples. This work was focused on chocolates and pralines production: industrial and hand-made processing of chocolates involves a contact of the product with plastic material. Previous studies on simulants under similar conditions of time and temperature reported migration of plastic additives[2], products of polymer degradation, colouring agents and non-intentionally added substances (NIAS)[3]. An important point regards the discover of oligomers of bisphenol A that could derive by unreacted species or polymer degradation. Their occurrence should be considered of high concern since they could be hydrolyzed *in-vivo* generating several bisphenol A units. Experiments were performed on three different chocolate types (dark, white and milk chocolate) and two simulant solutions (isooctane and ethanol) placed in contact with plastic objects of different shape and color. Migration tests were carried out in a climatic chamber and repeated three times according to the procedure described in legislation[4]. Since the degree of damage of the plastic surface is an important parameter affecting the migration, samples of different age, and submitted to different washing cycles, were examined. Analyses were performed by UHPLC-HRMS nano-system operating in capillary-flow mode coupled to a Q-Exactive mass spectrometer. Data dependent acquisition mode for targeted and untargeted analysis was exploited[5]. Results showed that old and new objects released different compounds. Kinetic curves were built by analysis of samples put in contact with polymeric materials for different time periods. Besides, a slight difference in the amount of oligomers released was found depending on the chocolate type. The possible correlation between age, degree of damage, and compound migration was explored.

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A Simple And Rapid GC-MS/MS Method For The Determination Of Phytoestrogens In Soy Drinks

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Phytoestrogens are a family of nonsteroidal natural compounds, with the function of secondary metabolites in many plants, such as soy, legumes, alfalfa and hop. They are considered endocrine disruptors chemicals, since they can interact with the human endocrine system, through a range of mechanisms which have not been completely elucidated so far[1]. Because of these complicated mechanisms of action, the effect of phytoestrogens on humans can be detrimental or beneficial, depending on numerous factors, such as age, habits, exposure to other chemicals. In the recent years, many vegetal food, based on soy and legumes, are spreading in the market and this could cause an excessive consumption of phytoestrogens. For this reason, it is important to determine the concentration of phytoestrogens in this kind of matrices, to estimate the intake of these substances.

In our lab, we are developing and testing different chromatographic techniques, coupled to mass spectrometry, for the analysis of phytoestrogens in food, especially soy based products. HPLC-MS is usually preferred for the analysis of phytoestrogens[2], since the GC analysis requires a derivatization step[3]. Nevertheless, GC-MS is a much more affordable technique and so we developed a method for the determination of five common phytoestrogens, biochanin A, daidzein, genistein, formononetin and coumestrol.

The derivatization procedure was deeply studied, testing different derivatizing reagents and performing an experimental design to find the optimal conditions of the derivatization reaction with *N,O*-bis-(trimethylsilyl) trifluoroacetamide (BSTFA). The results showed that a derivatization of the analytes in the injection port was possible, making the analysis more rapid and simple. On the other hand, various instrumental parameters, related to the tandem MS detection, were optimized to enhance sensitivity and specificity of the analysis.

The final method consisted of the addition of the derivatizing reagent and immediate analysis by GC-MS/MS, with injection performed with a programmed temperature vaporizer (PTV). Low detection limits (0.1-17 µg L^{-1}) and high specificity were obtained for all phytoestrogens; calibration curves were built in the range of 8-500 µg L^{-1} and good precision (relative standard deviations of 4.7-6.1%) was reached for formononetin, daidzein and coumestrol. Some problems arose with biochanin A and genistein, whose derivatives exhibited low stability; consequently, the determination of these two phytoestrogens was characterized by low repeatability. The optimized method was tested on soy drink samples from a local drugstore: a simple solid phase extraction was performed on soy milks and the extracts were derivatized and analyzed. High concentration (4-16 mg L^{-1}) of daidzein and genistein were found, while the other phytoestrogens were in the range of 20-400 µg L^{-1}.

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Milk Authenticity By Ion-Trap Proteomics Following Multi-Enzyme Digestion

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The practice of adding adulterating substances (water, starch, sodium citrate, urea, sucrose, melamine, etc) in milk products in order to raise profits is worldwide employed. In addition, higher priced milk, coming from minor dairy species, such as goat and buffalo showing high nutritional and economical value, is often illegally integrated with the lower priced cow milk. The presence of species-specific proteins, different from those declared in label, may be a serious problem for people with allergies. The development of proper analytical methods is therefore essential to protect consumer benefits and product authenticity, as well as to detect economic frauds.

Several analytical techniques, including capillary electrophoresis[1], polymerase chain reaction[2], enzyme-linked immunosorbent assays[3] and two-dimensional gel electrophoresis (2-DE)[4], have been used to evaluate the content of mixtures of milk of different species

In this study, a proteomic approach for the detection of adulteration processes of specific milks in mixtures is proposed. Milk samples digested by trypsin and chymotrypsin have been analyzed by nanoLC-ESI-IT-MS/MS. A post-database processing was performed to obtain the sequence assignments[5]. In the complex overview of the strategies reported for the evaluation of milk authenticity, our approach is characterized by reduced instrumental costs and analysis speed. Indeed, the proposed method is quite rapid, with the only exception of the digestion step that, however, is usually performed overnight, and then does not represent a real extra-time of analysis. Moreover for the protein characterization few microliters of sample are required and directly analyzed by a single shot-gun analysis, without a previous separation. In addition to a direct evaluation of milk traceability at low level of adulteration, this method has confirmed the identification of two effective signature peptides related to bovine milk source (LSFNPTQLEEQCHI and HQGLPQEVLNENLLR from β-lactoglobulin and αS1 casein, respectively) allowing the detection of hidden allergens at adulteration levels down to 1%.

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Analytical Pyrolysis With In-Situ Derivatization, Py(HMDS)-GC/MS Of Condensed And Hydrolysable Tannins: A Flexible Characterization Technique

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Tannins are natural oligomeric phenols. They are fundamental materials for the food and leather industries, and are widely studied for their biological activity. Tannins can be extracted from several types of lignocellulosic biomasses, and so their study is also of great relevance in the field of biomass conversion.

The main challenge in the analysis and characterization of tannins is their wide compositional variety. Wet chemistry methods such as colorimetric assays can only provide information on the total content, with little to no means of speciation. Liquid chromatography techniques (TLC, HPLC) have been used to characterize both hydrolysable and condensed tannins, but provide poor results in terms of peak shape and resolution[1]. The use of analytical pyrolysis techniques in the study of tannins is less well documented[2], but such techniques seem to be promising in this field. Both solid and liquid samples can be analysed without pre-treatment steps, and speciation could be possible by the analysis of their pyrolysis products. In the present work, Py-GC/MS was used to identify the main pyrolysis products of both hydrolysable and condensed tannins, starting from the analysis of their building blocks (gallic acid, ellagic acid, catechin). Moreover, the use of in-situ derivatization using hexamethyldisilazane (HMDS) as derivatizing agent allowed us to improve the quality of the results in terms of peak shape and resolution. Thermal degradation mechanisms were also investigated by performing experiments at different pyrolysis times. The results were used to characterize natural tannin extracts. This work shows the first results and the potential of a widely applicable technique for the analysis of vegetable tannins.

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A New Method for The Determination Of Crocins As Markers Of Quality And Authenticity By UHPLC-MS/MS

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Saffron is a precious spice known as "red gold" obtained from the dried stigmas of the *Crocus Sativus* L. a plant of the Iridaceae family. Its importance is related to the peculiar quality and sensory properties, in particular aroma, colour and taste that are mainly due to the presence of safranal, crocins and picrocrocin, respectively.

Crocins are water soluble-carotenoids of saffron and they are glycosyl esters of crocetin with different sugar moieties, such as glucose, gentiobiose, neapolitanose or triglucose; they can be present in saffron in cis- and trans- isomeric forms[1]. They are known to exert various pharmacological effects on various illnessand anti-tumor effects by inhibition of cell growth[2]. When saffron is exposed to white fluorescent light, trans isomers change into cis ones[3].

The aim of this study is to determine the content of crocins isomers of saffron of different origin and obtained by different process conditions and to use the cis-trans content ratio as index of quality and authenticity.

Analysis was carried out by UHPLC-MS/MS. A preliminary step of the study implied the optimization of the extraction efficiency for both crocins. An experimental design plan was set including independent variables: extraction solvents (MeOH, H_2O, MeOH/ H_2O with different ratio), time and temperature of Ultrasound Assisted Extraction (USAE).

The best conditions found were: saffron powder (5 mg) were extracted with 1.25 mL MeOH/H_2O (50:50, v/v) for 15 minutes in ultrasonic bath at T=25°C (Fig.1).
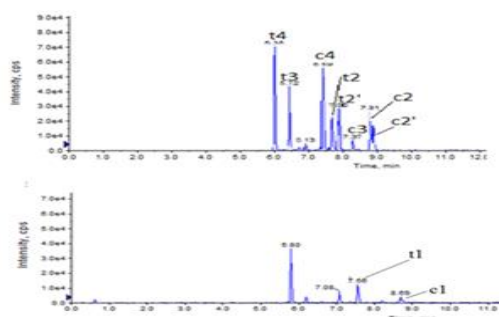
Extracted ion currents (XIC) of the selected transitions obtained from the analysis of a saffron extract

Results have been processed by Principal Component Analysis (PCA). This study is confirming the feasibility to study crocins isomers as marker of quality and authenticity of saffron samples.

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Panoramic Overview on the Enantioselection Performance of Inherently Chiral Surfaces: a Comparison between Systems with Different Atropisomeric Cores and Stereogenic Elements

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Enantiorecognition is a key issue in advanced analytical chemistry, particularly concerning the biological and pharmaceutical field. Enantiomeric molecules, being mirror-image structures, have identical physico-chemical scalar properties, but opposite pseudo-scalar ones. When interacting with a racemic probe, chiral molecules can recognize the enantiomers through diasteromeric interactions. Similarly, chiral electrodes are required for enantioselective electroanalysis, and the development of "intelligent" electrodes capable of discriminating enantiomers, in particular molecules of biological and pharmaceutical importance, remains as one of the major challenges in electroanalysis. We have recently proposed the first synthetic inherently chiral electrode surfaces able to neatly discriminate as separate peaks (in terms of potential values) the antipodes of model chiral probes, also drugs, both as enantiopure and racemate[1,2]. We have also verified the general validity of the inherently chiral concept, which does not depend from the chemical nature of the atropisomeric scaffold, testing chiral surfaces electrooligomerized from starting monomers with different molecular design (*i.e.* bithiophene, bibenzothiophene, biindole and paracyclophane cores) and different stereogenic elements (stereogenic axis *vs* helix *vs* plane). In order to fully elucidate the enantioselection capability of all of these heteroaromatic systems we propose a detailed comparison (an example in Figure) of our inherently chiral surfaces with different atropisomeric core *vs* thiahelicene-based films *vs* "two floor" paracyclophanic oligomers.
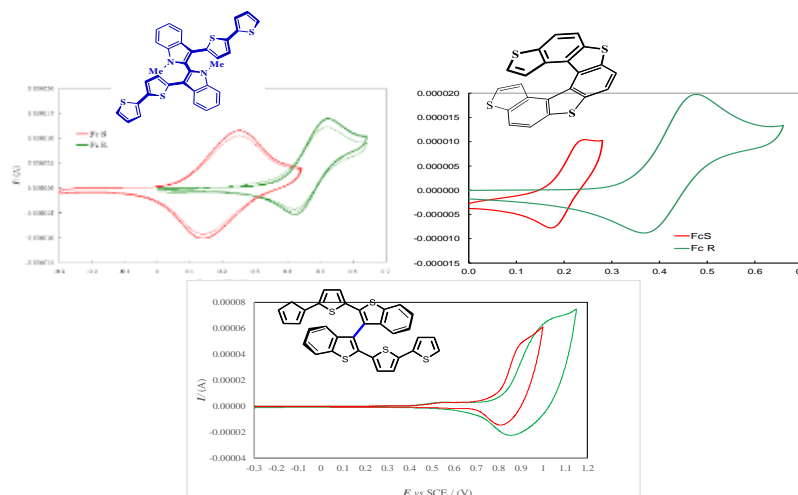
Figure. Enantioselection properties of inherently chiral oligomers with biindole, thiahelicene and bibenzothiophene units towards chiral ferrocenylic probes.

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Direct Electrodeposition Of Gold Nanoporous (Aunps) Structure Onto Gold Electrode As Potential On-Line Biosensor Based On D-Fructose Dehydrogenase (FDH)

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In the last decades, nanomaterials have shown great advantages in terms of functional properties for a wide range of technological applications[1]. Metal nanoparticles provide a lot of advantages compared to macroelectrodes, such as enhancement of mass transport, catalysis, high effective surface area and control on the electrode conductive microenvironment. Gold nanoporous (AuNPs) can be synthesized by using a chemical approach and electrodeposition. The main advantages of the electrodeposition method are the thickness, roughness and size control of the AuNPs layer[2]. In this work AuNPs was directly electrodeposited onto a gold electrode (AuE) by using a two-steps method: 1) sweeping the potential in a 10 mM $HAuCl_4$ solution (supporting electrolyte 2.5 M NH_4Cl); 2) applying a potential -3 V vs. $Ag|AgCl_{sat}$[3,4]. Initially we optimized the different parameters that can affect the electrodeposition process, such as scan numbers, concentration of precursor solution, etc. Afterwards, the nanostructured electrodes morphology was studied by scanning electron microscopy (SEM). The so modified electrodes were characterized with $Fe(CN)_6^{3-/4-}$ by cyclic voltammetry and electrochemical impedance spectroscopy, and compared to the naked gold electrode (AuE) in order to determine the electroactive area (A_{EA}), electron transfer rate constant (k^0), real surface area (R_{SA}) and the roughness factor (ρ)[5]. The electrode was further modified by thiols with different aliphatic or aromatic structure, length and functional groups (e.g. -COOH, $-NH_2$, -OH) in order to provide a different charge onto the electrode, which may affect the orientation of *Gluconobacter japonicus* Fructose Dehydrogenase (FDH). D-Fructose dehydrogenase (FDH; EC 1.1.99.11) from *Gluconobacter japonicus* NCBR 3260 is a heterotrimeric membrane-bound enzyme complex with a molecular mass of ca. 140 kDa, consisting of subunits I (67 kDa), II (51 kDa), and III (20 kDa). Subunits I and II have a covalently bound flavin adenine dinucleotide (FAD) and three heme C moieties, respectively. FDH shows strict substrate-specificity to D-(-)-fructose and is utilized in diagnostic and food analyses. FDH provides large catalytic current density in DET bioelectrocatalysis. However, the electron transfer pathway is going from the sugar oxidation through the three heme C moieties, which exhibit different formal potential[6]. Finally, the electrode which showed the best results in terms of kinetic and analytical properties was tested for the detection of D-(-)-fructose in fruit juice samples as potential on-line biosensor for food industrial production processes[7].

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Bioelectrochemical Characterization of ATG-Fructose Dehydrogenase (FDH$_{atg}$) from *Gluconobacter japonicus*: The Influence of Divalent Cations and Ionic Strength on Enzymatic Activity, Structure and Catalytic Current

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In the last decades, many redox enzymes and proteins have been investigated for the possibility of a direct electron transfer (DET) reaction between the active site of the enzyme and an electrode. DET reactions have received considerable attention for construction of biosensors and biofuel cells[1]. *Gluconobacter japonicas* fructose dehydrogenase (FDH) is a heterotrimeric-membrane-bound enzyme with DET characteristics. FDH catalyzes the oxidation of D-fructose to 5-keto-D-fructose. Subunit I (67 kDa) and II (51 kDa) comprise the substrate active flavin adenine dinucleotide (FAD) and additionally three heme c moieties as redox prosthetic groups, whereas subunit III (20 kDa) contains no cofactor and is presumed to be related to the stability of FDH[2]. In this work, the effect of Ca^{2+} and Mg^{2+} as well as ionic strength on the catalytic current of FDH modified electrodes were investigated by flow injection amperometry by drop-casting FDH onto graphite electrodes. Additionally, the activity of FDH was spectrophotometrically assayed by using both mono- and di-electron acceptors (ferricyanide, cytochrome c, and 2,6-dichloroindophenol) in the absence and presence of Ca^{2+} and Mg^{2+} at different ionic strength values. As FDH is a membrane-bound enzyme, so far impossible to crystalize, circular dichroism spectra were carried out under the same experimental conditions in order to verify possible structural changes in the secondary structure of FDH[3], allowing further considerations on the mechanism of DET.

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Thermodynamic Study Of The Interaction Of NTA-Phosphonate Derivatives With The Main Cationic Components (H^+, Na^+, Mg^{2+} And Ca^{2+}) Of Natural Fluids.

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Aminophosphonates represent an important class of biologically active compounds, infact they are structural analogues of the corresponding aminoacids. They are widely used as corrosion inhibitors and they are effective chelating agents that tightly bind metal ions, preventing them from forming insoluble precipitates. Despite these molecules are very important in the industrial fields, only few papers deals with information about their acid-base properties in aqueous solutions[1]. In this paper, three molecules were studied: a) N-phosphonomethyliminodiacetic acid (NTAP), b) N,N-Bis(phosphonomethyl)glycine (NTAP2) and c) Nitrilotris(methylenephosphonic acid) (NTAP3). NTAP is an important intermediate for the production of Glyphosate, whose production is very high, being the most produced reaching 90,000 tons/year. NTAP2 is a plant growth regulator, affects chloroplast membrane proteins and increases sucrose levels in sugarcane and to cause chlorosis in maize. NTAP3 is used as power plants and for the prevention of the formation of calcium carbonate scale. Therefore, we focused our attention on the determination of the protonation constants in three different ionic media as NaCl, $(CH_3)_4NCl$ and $(C_2H_5)_4NI$ using ISE-H^+ potentiometry. The measurements at different temperatures, namely T = 283.15, 298.15 and 318.15 K, were performed only in $NaCl_{(aq)}$. At infinite dilution and at 298.15 K, the refined values of the first protonation constants are: = 11.41±0.01, 12.39±0.02 and 13.27±0.03 for NTAP, NTAP2 and NTAP3, respectively. The enthalpy changes relative to the first protonation step at infinite diluition and at 298.15 K are: -15.7±0.3 kJ mol^{-1}, -20.5±0.4 kJ mol^{-1} and -33.5±0.2 kJ mol^{-1} (same order). The dependence of protonation constants on ionic strength was studied by means of a Debye-Hückel type equation and the SIT (Specific ion Interaction Theory) approach. Solubility measurements were carried out only for NTAP in the three ionic media at 298.15 K, in order to determine the total solubility and the solubility of the neutral species, as well as the Setschenow and activity coefficients. In addition, the formation constants between the ligands studied and Ca^{2+} and Mg^{2+} were determined at I = 0.15 mol dm^{-3} in NaCl at 298.15K.

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Securing And Ensuring Sustainable Use Of Red Grape By-Products: Recovery Of Polyphenols And Assessment Of Their Antioxidant And Antitumoral Activities With Highly Predictive Human Cell-Based Biosensors

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Recently, there has been great social and environmental interest for the efficient reuse of agricultural industry residues. In fact, agriculture and food processing industry generate a huge amount of waste streams, as well as co- and by-products, that are often not properly taken care of, both in environmental and economic terms. The present work aimed at the recovery and characterization of polyphenolic compounds extracted from red grape pomace (Vitis vinifera L.), a winemaking by-product. Polyphenolic compounds of dried red pomace were recovered by different extraction methods (ultrasonic-assisted extraction UAE, Naviglio®-assisted extraction NAV and supercritical fluids technique SFE). A significantly amount of anthocyanins, above all malvidin-3-O-glucoside, was recovered from dried red pomace with UAE and NAV extractions. The best extracts contained high amounts of anthocyanins were explored to determine antioxidant and anti-cancer activities. We developed an *in vitro* chemiluminescent bioassay to determine xanthine oxidase (XO) activity, one of the major sources of ROS in living endothelial cells. Intracellular XO activity was measured in 5×10^3 human endothelial cells in less than 20 min with a luminol/catalyst-based chemiluminescence assay with a limit of detection of 0.4 µU/mL. Moreover, the IC50 value of oxypurinol, the active metabolite of the standard inhibitor drug allopurinol was determined; oxypurinol addition (ranging from 5.0 to 0.5 µM) caused a linear decrease in XO activity, with an IC50 of 1.0 ± 0.5 µM[1]. The extracts derived from UAE and NAV were measured (ranging from 10 to 0.0 ug/ml), obtaining an IC50 of 0,86±0,07 µg/mL and 1,27±0,08 µg/mL, respectively. Then, we investigated the antitumoral properties of both extracts in two highly predictive human cell models, leukemia and breast cancer, using a spectrophotometric approach with a new tetrazolium salt (WST-8) in respect to a standard cell counting method. WST-8 is a highly soluble tetrazolium salt in water, reduced to yellow formazan by the activity of intracellular dehydrogenase. The amount of formazan generated in the cells is directly proportional to the number of living cells (λmax450 nm). After 72h of incubation, UAE and NAV extracts (ranging from 50 to 0,1 µg/mL) exhibited a GI50 (50% growth inhibition) in human breast cancer cells of 28.2 ± 1.2 µg/mL and 16.1 ± 0.9 µg/mL, respectively, while in leukemia cells 6.9 ± 0.1 µg/mL and 6.9 ± 0.1 µg/mL and 4.0 ± 0.2 µg/mL, respectively. The doxorubicin antineoplastic drug (ranging from 10 to 0,1 µM) was used as standard, showing a GI50 of 0.13 ± 0.07 µM in breast cancer cells and 0.76 ± 0.09 µM in leukemia cells. The results support the possibility of exploiting the extracts coming from grape processing by-products as ingredients for functional and innovative products in the nutraceutical, pharmaceutical or cosmetic fields.

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Characterization and shelf-life evaluation of gluten-free biscuits enriched with Chestnut flour

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Manufacturing of gluten-free products is usually characterized by the need of optimizing specific technological processes aimed at obtaining properties to fulfill consumer expectations, since the raw material used often gives poor results in terms of taste, aroma, consistency and nutritional properties. For these reasons, lately, different alternative ingredients are being tested with the aim of improving the final result.

Chestnut flour is a promising ingredient in this field, containing high quality proteins with a very good content of essential amino acids (4-7%), fibers (4-10%), and low fat (2-4%). Besides, it also contains vitamins and microelements[1].

It has been successfully used in few cases for bread and pasta production. Nevertheless, few data are at present available regarding the technological characterization and the shelf-life of chestnut based products[2].

This work was aimed at the optimization of a recipe for the production of gluten-free biscuits. Different samples were prepared by mixing chestnut flour and gluten-free flour in different proportions. Obtained products were evaluated by analysis of chemical-physical properties, colorimetric measures and sensory analysis. The behavior of the biscuits during 60 days of shelf-life has also been monitored.

Since oxidative stability is an important parameter affecting food shelf-life, the product was submitted to analysis by Oxitest (VELP Scientifica, Italy), a reactor based on the use of high temperature and over-pressure of oxygen allowing to easily measure a sample oxidative stability by accelerating the oxidation process.

Obtained data showed good and promising results since the increase of percentage of chestnut flour in the dough lead to a product with improved colour, harder texture, better taste and enhancement of oxidative stability, opening new perspectives in the use of chestnut flour for preparation of gluten-free products.

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Pseudo-ELISA Assay Based on Molecularly Imprinted Nanoparticles for the Determination of Hepcidin.

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Robust synthetic receptors able to recognize a target analyte of clinical relevance and prone to integration into sensing and assay platforms are an open challenge in the analytical panorama. In order to meet these requests, we exploited the technology of Molecularly Imprinted Polymer (MIP), i.e. a template-assisted synthesis in which monomers are polymerized in the presence of the analyte of interest, called template, to create cavities onto the growing polymer, which possess geometrical and chemical complementarity towards the template itself, in similarity to biological receptors[1,2]. Since MIPs exhibit stability to harsh conditions, low cost of production, ease of integration to electronics and tailor made selectivity, they could represent the alternative to antibody in immunoassays[2-5].

Here, we aimed at targeting Hepcidin, which is the main regulator of iron homeostasis[6] and has important clinical implications[7] but, given its tightly packed 3D-structure and its high conservation over the evolutionary ladder, its dosage proved to be not trivial for decades. Therefore, we exploited Hepcidin-addressed MIP nanoparticles (MIP-NPs)[8] to develop a pseudo-ELISA assay.

The MIP-NPs were synthesized to 20-60 nm in diameter; the functional characterization indicated the ability of MIP-NPs to selectively recognize Hepcidin with high affinity (low nM). The deposition of MIP-NPs onto the microplates and the protocol for Hepcidin measurement were considered and optimized step-by-step, achieving a range of response of 1-100 nM and LOD of 0.1 nM. Serum samples spiked with known Hepcidin concentrations (1, 10 nM) were used to evaluate the matrix effect on the response of the pseudo-ELISA.

In conclusion, our results highlighted both the MIP-NPs potential in terms of robustness, affinity and detection limits, and the limitations concerning the deposition protocol and the matrix effect, thus contributing to the MIP-based pseudo-ELISA development.

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A New, Rapid Bioluminescent Yeast Reporter Gene Bioassay For Quantitative Estrogen-Like Endocrine Disruptors Bioavailability Detection.

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Living cells (bacteria, yeast and mammalian cell lines) have been widely exploited for biosensing as they provide useful information about the bioavailability of target analyte[1]. Nonetheless, one of the main drawback of cell-biosensors based on reporter gene technology is the long assay time (hours) due to the slow RNA production of the reporter enzyme upon induction and its accumulation to achieve the needed detectability. Here we report the development of a improved bioluminescent yeast-based whole-cell bioassay which provide a fast (15 min) and sensitive detection of estrogenic compounds in 96-well microplate format. In particular, *S. cerevisiae* cells were genetically engineered to express the human estrogen receptor α (hERα) and the Nanoluc luciferase in response to compounds able to activate the estrogen signalling pathway. Nanoluc luciferase was used as BL reporter since it represents the brightest luciferase available to date (150-fold brighter than firefly luciferase), emitting at 460 nm, using the coelenterazine analog furimazine as BL substrate. In addition, its small size (only 19KDa) and the absence of post-translational modifications and disulphide bonds, enable rapid synthesis and folding of the reporter thus reducing response time. The Nanoluc luciferase coding sequence was further optimized to increase its expression efficiency in yeast cells, and the use of a destabilized variant, allowed to reduce the background expression obtaining a rapid response upon induction by target analyte. The estrogen bioassay is performed in 96-well plates by incubating 90μL of yeast culture ($1,5 \times 10^6$ cells/well) with 10μL of sample at room temperature (25°C) for 15 minutes, (compared with 2.5h of previously published yeast estrogen assays) making it the fastest BL yeast biosensor developed to date. Luminescence measurements are then performed with a luminometer without washing steps, by simple addition of 50μL of a custom BL substrate containing 10μM furimazine in a yeast-specific lysis buffer (Y-PER), optimized to provide a glow-type emission. The developed yeast bioreporter respond to 17-beta estradiol (selected as model analyte) with a limit of detection of 0.03nM and an EC50 of 1.5nM. The biosensor response to estrogenic compounds (e.g. diethylstilbestrol) and to several endocrine disruptors (i.e. bisphenol A, F and S) in spiked water samples is reported. In the perspective to apply the developed yeast bioreporters for actual on-field analysis, we also integrated yeast cells with a smartphone-controlled GoPro Hero5 camera as light sensor. To this end a 3D printed adaptor was fabricated to hold the GoPro camera and to house a custom 3D printed multi-well cartridge containing immobilized yeast bioreporters. This configuration combines the use of standardized sensitive detector with smartphone functionalities (i.e. connectivity, data elaboration, geo-tagging and the possibility to develop custom App), resulting in a versatile, user-friendly biosensing platform. Preliminary results obtained with the integrated device are presented.

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Synthesis And Analytical Characterization Of A Dual-Acting Pt(Iv) Prodrug With Potential Antitumor And Anti-Inflammatory Activities

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Pt(IV) complexes are organometallic antitumor prodrugs, activated *in vivo* to Pt(II) complexes with an irreversible reduction mechanism by endogenous biomolecules (glutathione, ascorbic acid)[1]. Reduction potential of those Pt(IV) complexes varies depending on axial ligand; specifically, it has been found that optimal values for in vivo reduction are obtained when platinum axial positions are functionalized with carboxylate groups[2]. In this work, the complex *cis-trans-cis*-[PtCl$_2$(*RS*-Ibuprofen-H)$_2$(*cis*-1,4-DACH)] was synthesized to obtain a potential prodrug having both antitumor and anti-inflammatory action. This can be achieved functionalizing both the axial positions of [PtCl$_2$(*cis*-1,4-DACH)] (kiteplatin) with two ibuprofen molecules. Ibuprofen was extracted from Brufen® commercial expired tablets, kiteplatin was synthesized as reported in the literature[3]. The synthesis *cis-trans-cis*-[PtCl$_2$(*RS*-Ibuprofen-H)$_2$(*cis*-1,4-DACH)] was carried out by adapting the procedure reported in (4,5). *Cis-trans-cis*-[PtCl$_2$(*RS*-Ibuprofen-H)$_2$(*cis*-1,4-DACH)] and its precursors were characterized by multinuclear NMR analysis and x-ray photoelectron spectroscopy (XPS) to determine Pt chemical oxidation state. The complex was electrochemically characterized by cyclic voltammetry to evaluate the possibility of reduction. in vivo. A reduction potential of -0.93V was found, whose value is compatible with *in vivo* reduction and consistent with analogous Pt(IV) complexes reported in literature (2). Perspective studies will be focused on the evaluation of *in vitro* cytotoxicity against human cancer cell lines.
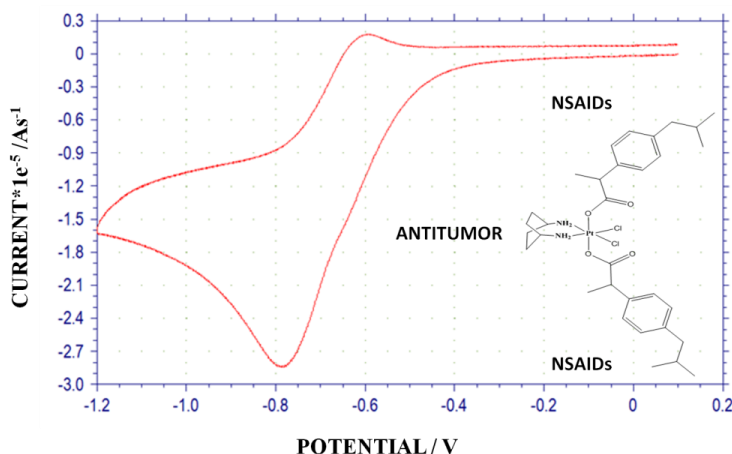
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Deposition of Palladium particles on glassy carbon surface modified by monolayer choline film and its electrochemical characterization

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The properties of palladium group metals have made them an invaluable source of active materials with a wide range of electrochemical applications and the definition of adequate deposition procedures of modulated-size nanoparticles is of key importance in many fundamental scientific and technological contexts[1,2]. Thus, deposition strategy of these active metals plays a fundamental role on the final properties of the prepared material. Choline is an essential nutrient for cell membrane function[3], but is also widely applied to metal and/or alloy electrodeposition procedures[4,5,6], where choline can be covalently grafted on glassy carbon surfaces using cyclic voltammetric methods (CV), forming stable cationic monolayer-modified electrode (GC/Ch). In particular, thick pure Pd particles were useful deposited from choline-palladium solutions without the addition of surfactant species and these resulting composite materials showed excellent properties on solderability and corrosion resistance[6]. In this study, a new electrodic material is based on the electrodeposition or adsorption of Pd ions on the GC/Ch electrode substrate. The choline film was electrodeposited on the GC surface by cycling the potentials between – 1.7 V and 1.8 V vs SCE; then Pd was electrodeposited by CV or directly adsorbed on the GC/Ch from acid solution containing K_2PdCl_6 1.5 mM. The effects of several experimental conditions such as: applied potentials, time of electrodeposition or adorption, choline concentration, etc. on the kinetics of Pd loading are considered and critically evaluated. The modified electrodes defined GC/Ch/Pd, were characterized by SEM and electrochemical techniques. The preliminary results confirm the presence of a globular and homogeneous dispersion of Pd particles on the GC/Ch substrate electrode with a considerable physical and chemical stability after prolonged electrochemical treatments. In addition, the study shows that the modified electrode GC/Ch/Pd exhibits a significant electrocatalytic activity toward oxidation of some compounds such as hydroxylamine, dopamine, epinephrine and common aminoacids in both acid and alkaline conditions.

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PQQ-Dependent Glucose Dehydrogenase On Modified Carbon Nanotubes: Polythiophene Supported Electron Transfer For Sensing Applications

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Conductive polymers are very attractive in the field of electrochemical biosensors. The intrinsic conductivity and the possibility to equip the chains with different functional groups by choosing the proper monomers make them very useful to improve the connection between the electrode and the redox center of an enzyme. In this context, polythiophene derivatives have only recently been employed in biosensing field in order to immobilize some enzymes like glucose oxidase, catalase or laccase. Anyway the use as conductive wiring matrix has not been fully exploited yet. Pyrroloquinoline quinone dependent glucose dehydrogenase (PQQ-GDH) has been extensively studied for biosensors and biofuel cells development thanks to its high catalytic activity and oxygen insensitivity. The redox active site is however embedded in the protein structure, so it is not easy to achieve an efficient direct electron transfer (DET). In this work, polythiophene copolymers (PTh) have been used as modifier for electrode surfaces in order to allow the immobilization of active PQQ-GDH and to simultaneously improve the direct electrical connection of the enzyme with the electrode. Polymer films were electrosynthesized onto carbon nanotubes (MWCNTs) modified gold electrodes from mixtures of 3-thiopheneacetic acid ($ThCH_2CO_2H$), 3-methoxythiophene ($ThOCH_3$) and, eventually, 2-thiophenesulfonic acid ($ThSO_3^-$) using a pulsed deposition method. Polythiophene deposition significantly improves the bioelectrocatalysis of PQQ-GDH. The process starts at -0.2 V vs. Ag/AgCl and allows glucose detection at 0 V vs. Ag/AgCl. Several parameters of the electro-polymerizing method were evaluated to maximize the anodic current output after enzyme coupling. Polymer deposition has been performed first in acetonitrile which required then a transfer into the aqueous system. However, electrosynthesis performed in an aqueous solution verified that also in this case successful electrode modification is feasible. Catalytic currents for the coupled PQQ-GDH have been found to be significantly higher. The electrode modifications were characterized by SEM, EDX, FTIR, XPS and UV-Vis. The obtained results suggest a further investigation of this kind of polymers and, in particular, the study of the interaction with other enzymes in order to employ them in building up biosensors and biofuel cells.
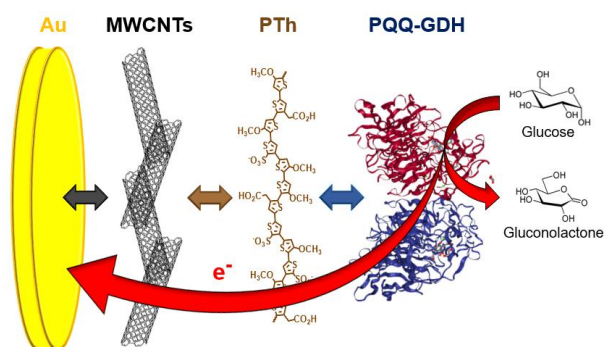
Amperometric Genosensor Based On PNA Probes Implemented On Carbon Nanotubes-Modified Screen Printed Electrodes

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Nucleic acid-based biosensors (genosensors) have received great attention in the past decade, nucleic acids being promising molecular probes due to the ease of functional modification, the specificity for base pairing, and the predictability for intermolecular or intramolecular interactions. In a research program dealing with the development of innovative sensors as powerful analytical tools for assessing food safety[1,2], we combined performance of DNA-mimic probes based on Peptide Nucleic Acids (PNA) with the enhancing properties of carbon nanotubes (CNTs) as binding substrates on Carbon Screen Printed Electrodes (CSPEs). The findings of our previous studies[3], focused on the use of the same PNA probes on all-gold SPEs, evidenced best results using a non-competitive approach, based on the binding of a target DNA ascribable to "Roundup-Ready (RR)" transgenic soy by a PNA-Capture Probe (CP)-functionalized

sensor, followed by the hybridization with a properly synthesized PNA-Reporter Probe (RP). The latter bears a biotin tag, capable of strong interactions with a streptavidin-alkaline phosphatase conjugate, which converts a substrate into an electroactive species. In this work, we experimented the use of CNT-modified electrodic platforms in order to reach higher loading capability of CP, if compared to gold substrates combined with Self-Assembled Monolayers from mercaptoundecanoic acid as linkers.
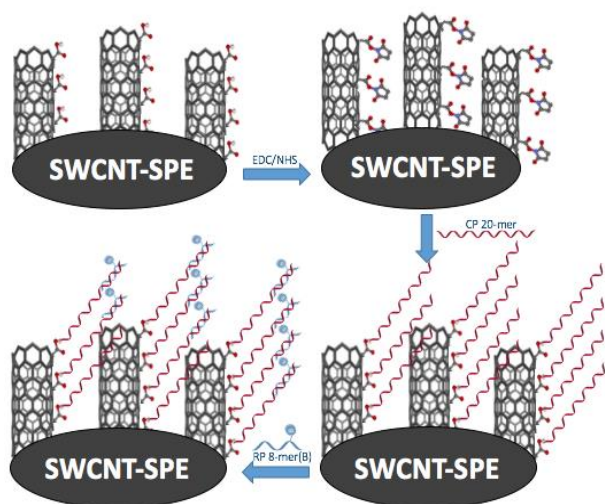
Another crucial aspect deals with the nature of the RP, since we noticed the formation of CP/Target-DNA/RP adduct when using PNAs both for capture and reporter probes. To overcome this limit, we moved to DNA-based RPs with the same oligonucleotidic sequence, obtaining encouraging results in terms of signal inhibition associated to hybridization of the target DNA up to nanomolar scale. Further studies currently ongoing are focused on the comparison of such inhibitive approach with the use of a sandwich-type assay based on the use of a longer DNA target, showing hetero-complementarity with CP and RP. Further improvements in terms of sensitivity enhancement could be also reached by conjugation of RPs with molecular nanomaterials such as dendrimers. The performance of polyamidoamine (PAMAM) dendrimers were already demonstrated in our previous studies concerning the development of competitive[4] and noncompetitive[5] immunosensors. These strategies will be further investigated to optimize the DNA assay protocols aimed at specific recognition of transgenic material at trace levels for GMO food labelling purposes.

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Selective Detection Of Halogenated Aromatic Hydrocarbons In Water With Piezoelectric Sensors Coated With Electropolymerized (2,2'bithiophene) Functionalized Cavitands

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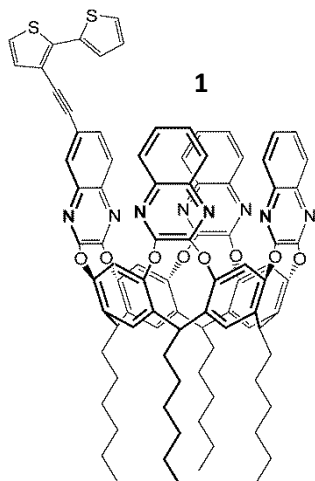
New sensing coatings for piezoelectric sensors developed on gold-coated quartz crystals for Electrochemical Quartz Crystal Microbalance (EQCM) were electrosynthesized and characterized. The monomeric receptor **1** unit consists of cavitands functionalized at the upper rim with quinoxaline moieties, one of which bearing the bithiophene functionality connected *via* an unsaturated spacer arm. Previous studies carried out during the design of the monomeric unit evidenced as the use of 2,2'bitiophene building blocks is a crucial aspect for the reactivity of the receptors toward the electropolymerization, since the parent cavitand functionalized in the same position with a single thiophene unit was not capable to undergo electropolymerization, due to reduced reactivity of the monomer and steric hindrance of the system[1-3].

The cavitand functionalized with 2,2'bitiophene monomeric unit presented excellent performance in terms of reactivity, regularity of the polymer growth as well as compactness of the layer deposited on the gold surface of the crystals. The electropolymerization process, carried out under potentiodynamic conditions by cyclic voltammetry, was highly reproducible, as shown by the frequencymetric control of the growth (Figure 1). Analyte *host-guest* inclusion in cavitands was evaluated by insertion of the polymer-coated piezoelectric quartz in a 100-µL flow cell connected to a peristaltic pump. Continuous dynamic flow experiments carried out with aqueous solutions of different chlorinated aromatic and aliphatic hydrocarbons evidenced a remarkable selectivity for halogenated aromatic hydrocarbons, with frequency shifts increasing with the chlorination degree of the benzenes. A less intense but significant interaction was also observed with saturated and unsaturated chlorinated aliphatic hydrocarbons, whereas non-chlorinated aliphatic molecules did not give raise to significant frequency shifts.
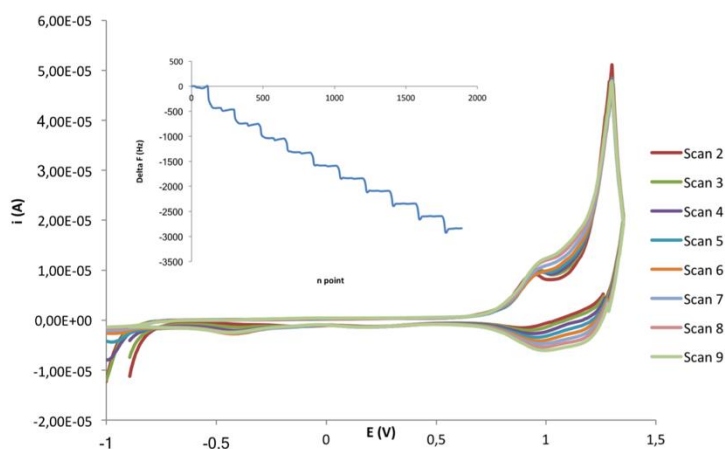
Figure 1: CV scans and frequencygrams (inset) recorded during the electropolymerization of the cavitand 1

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Innovative gold-free carbon nanotubes/chitosan-based competitive immunosensor for determination of HIV-related p24 capsid protein in serum

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In the last decade, the need for simple, rapid, sensitive, specific, and inexpensive screening methods for diagnosis of HIV infection led to focus the attention on the HIV1-related capsid proteins p24 [1]. This protein is expressed in the core of the viral cell and occurs in blood-circulation since the first few weeks after infection, making it an useful biomarker for early diagnosis purposes. In this work we developed and validated the first competitive electrochemical immunosensor for the detection of HIV-capsid protein p24 as a simple, easy-to-use and promising tool for screening of sample for early diagnosis of HIV infection. The immunodevice was implemented on disposable gold-free Single-Walled Carbon Nanotubes-functionalized Screen-Printed Electrodes (SWCNT-SPEs) to take advantage of CNT properties in terms of enhancement of electrochemical active area, more efficient immobilization of bio-receptors and improvement of the electronic transfer process, aimed to a signal enhancement. The competitive sensor[2,3] is based on the immobilization of the target protein on the electrodic surface, through chitosan/glutaraldehyde system, as simple linker able to ensure in mild conditions a robust immobilization and a good exposition of the p24 for the interaction with the mouse anti- p24 IgG1. The immunosorbed antibodies were

finally detected by an Alkaline Phosphatase-labelled Rabbit Anti-Mouse (RAM-AP) reading antibody. The immunosensor setup as well as the experimental conditions for the execution of the assay were optimized by means of a two-factor and three levels experimental design aimed to find the best concentration of the immunoreagents[4]. The competitive immunosensor exhibited good analytical performance in untreated human serum in terms of specificity, sensitivity and absence of matrix effect. No significant cross-reactivity from other serum markers, such as CA125 and CEA, was observed. The performance of the realized device, coupled with the advantages of an easy preparation, low-cost and portability of the instrumentation, makes it an analytically robust diagnostic tool valuable for implementation of large-scale screening programs for early diagnosis of seropositivity.
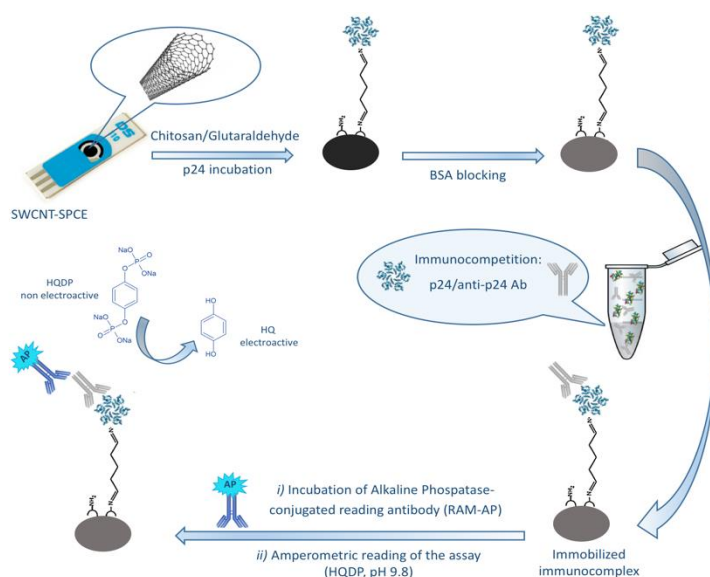
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Dynamic Spectro-Electrochemical Study of Pd in Fuel Cells with FEXRAV

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To fight the inexorable increasing of energy demand and pollution, it is crucial to develop highly efficient and clean energy conversion devices. In fact, current technologies have a bad impact on the environment or require great energy and raw material costs[1]. A winning approach is the controlled conversion of chemical energy to electricity from renewable sources[2]. For this reason, devices such as fuel cells are very interesting. In particular, ADAFCs (Alkaline Direct Alcohol Fuel Cell) are very promising because, in addition to producing high-power electrical energy and efficiency, they use cheaper catalysts than the commonly used platinum and are industrially attractive for the production of acetates.

These devices use a palladium-based catalyst, whose efficiency tends to decrease after hours of work. It is therefore essential to study the reaction mechanisms and behavior of the species on the electrode in order to increase the durability and preserve the efficiency of these devices. To achieve these results, it is important to understand how the Pd-based catalyst poisoning occurs to prevent it. Very important information is provided by the state of valence and structure of the catalytic material. Electrochemical and spectroscopic studies that are commonly conducted are unable to provide sufficiently accurate answers; for this reason, a relatively new technique has been used combining X-ray spectroscopy and dynamic electrochemistry: the FEXRAV (Fixed Energy X-ray Absorption Voltammetry)[3]. In our knowledge[3,4,5], this is the first documented use of FEXRAV on ADAFC to study the behavior of palladium and very interesting results have been achieved.

FEXRAV records variations in X-ray absorption and/or fluorescence intensity at a fixed energy, while the electrode potential varies. With the help of synchrotron light, it is possible to carefully follow the transition between the different valence states of the observed elements during the execution of classic electrochemical techniques: thus, FEXRAV results a very promising technique to study the evolution of electrocatalytic materials under operating conditions.

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Detection Of Organophosphorus Pesticides Using A DNA-Based Sensor

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In this work, we propose an electrochemical DNA-based sensor for sensitive multidetections of profenofos, an organophosphorus pesticide, based on a competitive format and disposable screen-printed arrays. To improve the sensitivity of the DNA-based sensor, polyaniline film and gold nanoparticles were progressively electrodeposited on the graphite screen-printed electrode surface by cyclic voltammetry[1,2]. Gold nanoparticles were then employed as platform for the immobilization of thiol-tethered DNA oligonucleotide sequence complementary to the DNA aptamer. Different profenofos solutions containing a fixed amount of biotinylated aptamer by DNA-based arrays were analyzed. Streptavidin-alkaline phosphatase conjugate was then added to trace the affinity reaction. The enzyme catalyzed the hydrolysis of 1-naphthyl phosphate to 1-naphthol. The enzymatic product was detected by differential pulse voltammetry. A decrease of the signal was obtained when the pesticide concentration was increased, making the sensor work as signal off sensor. Various experimental parameters of the realized DNA-based nanostructured sensor were studied and optimized using optical and electrochemical techniques. Under optimized conditions by testing key experimental parameters, a dose-response curve was constructed between 0.05-10.0 μM profenofos concentration range. The selectivity of the DNA-based sensor was also confirmed by the analysis of non-organophosphorus pesticides.

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Anchoring Of His-Tag-Rab7 Protein On Nanoporous Silicon. Toward Surface Imprinting For Optical Sensing Applications.

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Molecular imprinting is a universal concept to generate materials with "molecular memory" by performing a polymerization of suitable functional monomers in the presence of a target molecule acting as a template. The subsequent removal of the template creates recognition sites in the molecularly imprinted polymer (MIP) that can, further on, selectively rebind the target[1]. Although this concept proved to be successful in preparing selective sorbents for compounds of small molecular weight (~200–1200 Da), several specific problems can arise if the target is a macromolecular protein[2] as the classical imprinting methodologies fail to address the peculiarities of protein targets. The difficulties are largely attributed to the intrinsic properties of the proteins. Due to their fragility irreversible conformational changes may occur during polymerization; moreover, the large size of the proteins makes them difficult to remove from, or rebind to a highly cross-linked polymeric network. Among the approaches introduced during the past decade to overcome the barriers of protein imprinting[2], surface imprinting emerged as the main strategy for macromolecular imprinting. This approach restricts the formation of imprinted binding sites to the surface of a polymer or to a very thin polymer layer with thickness comparable to the size of the protein template. The immobilization of the protein through a self-assembled anchor layer offers additional advantages over polymerization from a protein-monomer mixture in terms of generating uniformly accessible binding sites. Herein, we propose a new method to prepare Rab7 protein oriented surface-imprinted nanoporous silicon photonic crystals (PhCs) with high template utilization efficiency. Nanoporous silicon PhCs are prepared by controlled electrochemical etching of silicon and subjected to thermal oxidation generating SiO_2 to aid surface modification[3]. A His-tag is used as the anchor to facilitate the protein immobilization/removal, by exploiting a 3-step protocol functionalization of PhC surface involving i) SiO_2 silanization with glycidoxypropyltrimethoxysilane (GLYMO) preliminarly reacted with iminodiacetic acid (IDA); ii) Ni^{2+} attachment by complexation with IDA; iii) Rab7 anchoring due to strong interaction between histidine residues and Ni^{2+}. Rab7 is used as target molecule as it is a small GTPase belonging to the Rab family with a key role on different cellular pathways and processes[4]: it is fundamental for lysosomal biogenesis, positioning and functions, and for trafficking and degradation of several signaling receptors. Furthermore, Rab7 has specific functions in neurons. Each step of PhC functionalization is monitored by Visible reflectance spectroscopy. Fourier transform of each spectrum affords a peak proportional to effective optical thickness (EOT) of the porous layer[3], which is used as analytical signal for monitoring successful functionalization event. Also, protein removal by EDTA and reversibility of functionalization process is checked by determining EOT values. The developed protocol for anchoring his-tag Rab7 protein represents the preliminary step of Rab7 imprinting process, which involves subsequent self-polymerization of dopamine to control the imprinted shell thickness, protein removal by EDTA and rebinding tests monitoring the optical behavior of MIP on PhC, including its imprinting efficiency and selectivity.

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A Novel Biosensor Based on Chemiluminescent Lateral Flow Immunoassay Integrated with Amorphous Silicon Photodiodes for Quantitative Detection of Human Serum Albumin

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Paper-based biosensors are emerging analytical tools for rapid on-site detection of clinical biomarkers in complex matrices, thanks to their ease of use and low cost. In this context, Lateral Flow Immunoassay (LFIA), a technology widely applied in resource-poor or non-laboratory environments, is based on prefabricated strips of a carrier material containing dry reagents that are activated by applying the fluid sample. LFIAs are mostly available for qualitative analyses exploiting naked eye observation of color signals due to a colloidal gold tracer. Recently, we reported LFIAs exploiting enzyme tracers and chemiluminescent (CL) detection to obtain quantitative information and reach high detectability[1,2]. Additionally, thin film hydrogenated amorphous silicon (a-Si:H) photodiodes[3,4] have been recently proposed as a low-cost and versatile technology for developing miniaturized biosensors with high degree of integration and providing high assay detectability and sensitivity. Herein, we report the development of a CL-LFIA-based biosensor with integrated a-Si:H photosensors array to quantitatively detect human serum albumin (HSA) in urine samples. The analytical method is based on an indirect competitive immunoassay using horseradish peroxidase (HRP) as a tracer and a luminol/enhancer/hydrogen peroxide cocktail for CL detection. The system is composed of a PDMS cartridge that houses the LFIA strip and all the reagents necessary for the CL immunoassay and that is aligned with an array of a-Si:H photodiodes deposited on a glass substrate. During the LFIA analysis, the photodiodes are employed to acquire the CL signals, through a custom portable electronics equipment. The method is simple and fast with a detection limit of 3 mg L^{-1}. It provides quantitative analysis in the range of $3\text{-}230 \text{ mg L}^{-1}$ which is suitable for measuring physiological levels of HSA in urine samples and their variation in different diseases. The analytical performances of this method were compared with those obtained using a charge-coupled device (CCD) as detector. The use of CL detection allowed accurate and objective analytes quantification in a dynamic range that extends from nanomoles to micromoles. Based on these results, it was demonstrated that the integration of the a-Si:H photosensors array with CL-LFIA technique provided a compact, sensitive and potentially low-cost microdevice for chemiluminescence and bioluminescence-based bioassays with a wide range of possible applications for in-field and point-of-care bio-analyses.

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Optimizing The Electrodeposition Protocol Of Enantioselective Inherently Chiral Electrode Surfaces: A Profilometry Investigation

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In our current research on enantioselective voltammetry on chiral electrodes based on electrodeposited enantiopure inherently chiral oligomer films, after achieving the first proof of concepts[1-5], we are currently concentrating on key issues such as optimizing the deposition protocol to enhance performances and improve reproducibility, as well as looking for interpretative/predictive guidelines concerning the observed enantioselection phenomena.

To this aim we are carrying out *inter alia* a systematic profilometry investigation of chiral film surfaces electrodeposited from two different chiral monomers according to a carefully optimized protocol, studying the effect of key experimental parameters such as the underlying electrode support, the deposition medium, the potential scan rate and the deposition cycle number, also comparing films obtained from enantiopure monomers with racemate ones.

The interesting results will be presented and discussed, also in connection with the electrode enantioselection performance.

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Potentiometric Sensor for Non Invasive Lactate Determination in Human Sweat

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The present work describes a non invasive lactate sensing in sweat during workout. The sensing system is based on a non-equilibrium potentiometric measure performed using disposable, chemically modified, screen printed carbon electrodes (SPCEs) that can be wetted with sweat during the exercise. The potentiometric signal, which is proportional to lactate concentration in sweat, is produced by a redox reaction activated by UV radiation, as opposed to the enzymatic reaction employed in traditional, blood-based measuring devices. The sensing system exhibits chemical selectivity toward lactate with linearity from 1 mM up to 180 mM. The dynamic linear range is suitable for measurement of lactate in sweat, which is more than 10 times concentrated than hematic lactate and reaches more than 100 mM in sweat during workout. The noninvasive measure can be repeated many times during exercise and during the recovery time in order to get personal information on the physiological and training status as well as on the physical performance.

The device was successfully applied to several human subjects for the measurement of sweat lactate during prolonged cycling exercise. The lactate concentration changes during the exercise reflected the intensity of physical effort. During the exercise sweat was simultaneously sampled on filter paper and extracted in water, and the lactate was determined by HPLC for method validation. This method has perspectives in many sport disciplines as well as in health care and biomedical area.

Antibody Powered Nucleic Acid Release Using A DNA-Based Nanomachine

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Inspired from transport proteins, highly evolved machines that are essential to the crucial mechanism of cell transport, we rationally designed here a new class of DNA-based nanomachines that can reversibly load and release a molecular cargo upon the binding to a specific target antibody. Our strategy to rationally design an antibody-driven DNA-based nanomachine involves the use of a triplex forming DNA strand that is designed to recognize a specific DNA strand through the formation of a clamp-like triplex forming mechanism involving both Watson-Crick and Hoogsteen interactions and which is conjugated at the two ends with a pair of antigens[1]. Antibody binding to the two antigen tags in the nanomachine causes a conformational change that energetically disrupts the triplex-forming Hoogsteen interactions in the triplex complex thus destabilizing the nanomachine/cargo complex. The design principle for antibody-powered release of a molecular cargo strand is highly generalizable and can easily be adapted to other antibodies via the expedient of changing the recognition element employed. We have demonstrated here that our approach can be extended to different triggering antibodies and the effect can be specific and selective enough even in complex media (90% serum).

Given these attributes, the antibody-poweered DNA nanomachines we have developed here may prove of utility in a range of applications, including point-of-care diagnostics, controlled drug-release and in-vivo imaging.

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Green Chemical Approaches toward High-Quality semiconductor thin film by Electrochemical Atomic Layer Deposition

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The Electrochemical Atomic Layer Deposition (E-ALD) is a green technique that allows to produce thin films of semiconductor. Exploiting Surface Limited Reactions (SLR), it enables the deposition of highly ordered ultra-thin films from diluted aqueous solutions and at room temperature and pressure. Underpotential deposition (UPD)[1] is a type of electrochemical SLR, where an atomic layer of a first element is deposited on a second, at a potential prior to (under) that needed to deposit the first element on itself, so that the resulting deposit is generally limited to an atomic layer. It occurs when the depositing element is able to somehow interact with the substrate, so that the deposition of the layer in direct contact with the substrate occurs at a potential preceding bulk deposition, that is, the deposition of the element on itself.

E-ALD enables the grow of ultra-thin films and 2D structured materials of interest in the fields of the nanoelectronics to new solar energy materials.

In the study presented here, E-ALD was used to grow transition metal chalcogenides. Central in the method is the necessity of singling out the proper electrochemical conditions which need to be used for deposition, namely potentials, reactants, electrolytes and pH. These are strictly dependent on the compound one wants to form on the substrate used.

The electrochemical conditions necessary to form ultra-thin films transition metal chalcogenides are described here and a miniaturized electrochemical flow cell. Here We presents also preliminary results of a structure characterization By SXRD at the ESRF.

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Tunable Growth Of Gold Nanostructures At PDMS Surface To Obtain Plasmon Rulers With Enhanced Optical Features

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Efficient coupling of plasmonic nanomaterials to optically transparent polymers still is a challenge in order to obtain affordable, versatile, and sensitive surface plasmonic devices. The *in-situ* fabrication of gold and silver nanoparticles on PDMS has been reported, but the resulting bulk sensitivities (of up to 70 nm RIU- ₁) may still be improved. The authors report that few simple modifications to the general preparation of these composites (AuNPs@PDMS) can result in substantial improvements of the optical features. A two-steps growth of AuNPs@PDMS is found to be particularly effective. It includes chemical treatment of the PDMS surface before the formation of well-exposed and densely-packed 3D conglomerates of gold spheroids with enhanced bulk sensitivity. Differently from available approaches, the structures obtained by this method display sensitivity to refractive index change of about 250 nm RIU-1. This is 3.5 times higher than spherical nanoparticles prepared by similar protocols and is near the optical performance of anisotropic NPs. Due to the strong 3D character of the structures, excellent plasmon coupling is realized on PDMS surface. The authors also show that these nanocomposite substrates can be subjected to external stimuli and then exhibit red shifts or blue shifts typical of induced plasmon coupling and uncoupling. Hence, the method represents a major step forward in terms of high- performance composite plasmonic nanomaterials for use in biosensing.

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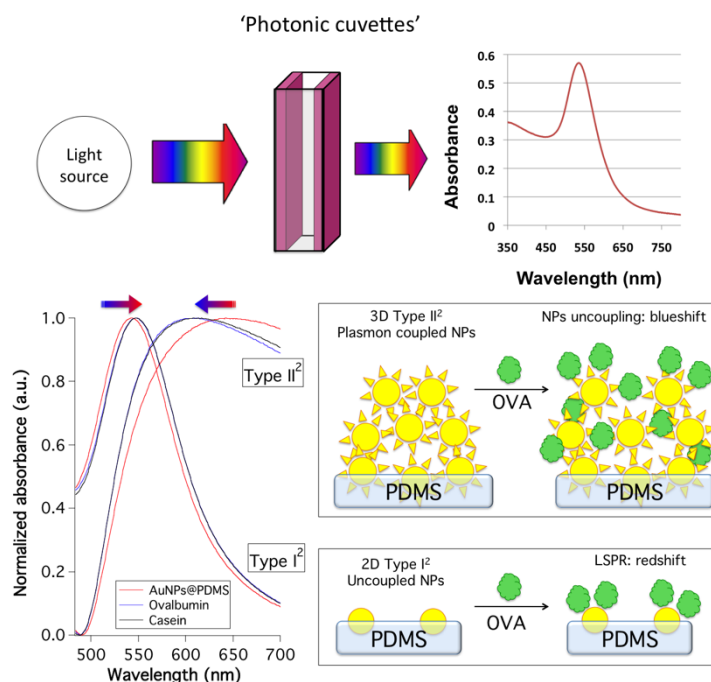
Synthesis of Xylitol-stabilized gold nanoparticles: a quantitative and sensitive method for xylitol detection in oral fluid by means of colorimetric assay

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Here we present the controlled synthesis of xylitol-stabilized gold nanoparticles (AuNPs) for the sensitive and reproducible detection of xylitol in oral fluid. The optical detection is achieved by following the early stage kinetic formation of gold nanoparticles at fixed wavelength by spectrophotometry. In fact, Xylitol in alkaline solution is able to induce the fast reduction of Au(III), which leads to highly stabilized and spherical AuNPs suitable for colorimetric assays.

Xylitol is an assessed anticariogenic agent, safe and effective in controlling dental caries[1]. Therefore, the detection of minimal salivary levels of this polyol along time is crucial to set up effective administration[2].

Traditionally, the quantitative detection of xylitol in saliva/oral fluid is carried out by commercially available enzymatic tests, cost and time expensive.

The sensitivity of the developed approach is comparable to enzymatic test, but allows to get quantitative results in minutes, in a cheap and green manner. The coefficient of variation is ~7% within the calibration range, i.e. 10-1 mg/mL, with an estimated detection limit of ~80 mg/L both in standard samples and in real oral fluid.
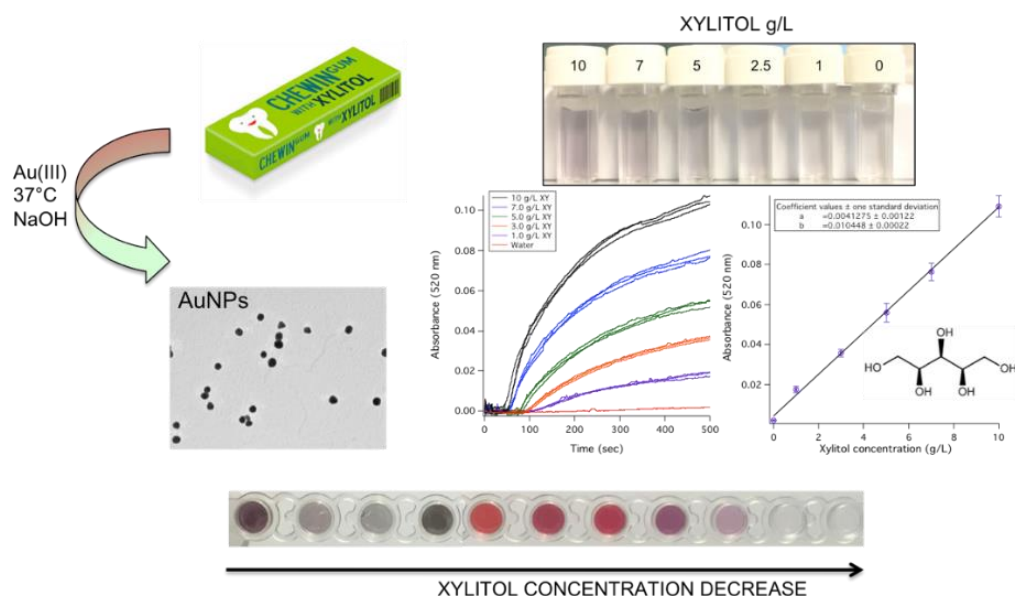
Ethanol Determination In Several Pharmaceutical Tinctures, Using New Enzymatic (Or Not Enzymatic) DMFC Devices And Comparison With A Conventional Catalase Biosensor, Considered As Reference Method.

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In previous research[1,2], we investigated the possibility to utilize Direct Methanol Fuel Cell (DMFC), both enzymatic or not enzymatic, for analytical purposes[1]. Our research group has now continued the analytical research, devoted to the use of this enzymatic and not enzymatic Fuel Cell. The research reported in this communication concerns the possibility to check, in a simple and inexpensive way, the ethanol content of several pharmaceutical tinctures using the enzymatic, or not enzymatic DMFC device. In this case the obtained results have been compared both with the ethanol content declared by the producer firms and with data obtained analyzing the same samples using an amperometric catalase enzyme sensor, recently pointed out in our laboratory[2], considered as reference method. The purpose of the present research was to determine the concentration of ethanol in five different mother tinctures for pharmaceutical use, by means of three different sensor methods: one using a classic amperometric biosensor, based on the catalase enzyme, already previously tested[3], and the other two, that use, for analytical purposes, a small direct methanol fuel cell (DMFC), however, one of the latter two, uses not only the DMFC cell, but also the alcohol dehydrogenase enzyme immobilized and inserted into the fuel cell. These three sensor methods were compared, from the analytical point of view, by determining the concentration of ethanol contained in five different mother tinctures for pharmaceutical use. First of all, the full analytical characterization of three sensors was performed. After this analytical characterization, five pharmaceutical tinctures, containing ethanol, purchased at common drugstores, were analyzed. The nominal values were provided by pharmaceutical companies. The results obtained were then processed with two statistical tests (F-test and t-test) to compare them from the point of view of precision (F-test) and "accuracy" (t-test). In conclusion, an application on real pharmaceutical samples was shown, for which the consistency between the values found experimentally with the "fuel cell" and the nominal values (provided by the pharmaceutical companies), generally obtained by official methods (titrimetric, chromatographic, etc.), has always proved to be good (as evidenced by the t-test). Also, the concordance among the values obtained with catalase enzymatic biosensor, nominal values and those found with the fuel cell has been satisfactory. Finally, the correlation between the enzymatic and the non - enzymatic "fuel cells" was also good, but the introduction of the enzyme alcohol dehydrogenase was positive, as it increased the calibration sensitivity of the method and, above all, it drastically decreased the response time and hence the time of individual measurement. In addition, the life-time of the non-enzymatic "fuel cell", in the conditions in which it was used, proved to be extremely long, compared to the life-time of a classical biosensor, such as catalase biosensor. While that of the latter was virtually the same as that of the "fuel cell" with the addition of the enzyme alcohol dehydrogenase. Conversely, the selectivity towards the different alcohols was better for the catalase enzymatic biosensor. Finally, the data obtained with three sensor methods in the applications on the real samples were mutually correlated, as demonstrated by the good values of the obtained R^2.

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A Smartphone-Based Biosensor for Ultrasensitive Chemiluminescent-Lateral Flow Immunoassay for the Quantification of Ochratoxin-A in Wine and Istant Coffee

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Ochratoxin A (OTA) is a mycotoxin produced by several species of *Aspergillus* and *Penicillium* fungi that is detected worldwide in various food and feed sources. Since OTA represents a potential hazard for human health, the European Community (EC) has established a maximum level for OTA in various feed and foods, in particular 2 μg L^{-1} in grape juices, wine and must and 10 μg L^{-1} in instant coffee. Several instrumental analytical methods are currently available for detecting these toxins in foodstuff, but they require complex sample preparation and dedicated laboratory equipment. Biosensors are very promising analytical tools for rapid on-site detection of analytes in complex matrices. We recently described a biosensor for multiplex detection of type-B fumonisins and B1 aflatoxin in maize samples based on a chemiluminescence Lateral Flow ImmunoAssay (CL-LFIA) coupled with a portable ultrasensitive CCD-based "contact" imaging device[1]. The use of CL detection allowed accurate and objective analytes quantification, down to picomoles, rather than qualitative or semi-quantitative information usually obtained employing conventional LFIAs based on colloidal gold labelling. Recently, thanks to the technological advance in complementary metal oxide semiconductor (CMOS) camera technology, smartphones are emerging as detectors suitable for CL-based bioassays[2]. Here, we report on the development of a smartphone-based simple, rapid and accurate biosensor based on CL-LFIA method for quantitative detection of OTA in wine and instant coffee. The biosensor is based on a direct competitive immunoassay employing horseradish peroxidase (HRP)-OTA conjugate as a tracer, which is detected by adding the luminol/enhancer/hydrogen peroxide CL cocktail and by using a smartphone's camera for image acquisition and data handling. A self-standing microfluidic cartridge was developed, which houses the LFIA membrane and all the reagents necessary for the analysis. For CL signal detection, a smartphone cover-like adaptor, containing a plano-convex lens aligned with the camera, was developed and produced by 3D printing. Once the operator has carried out the assay using the LFIA cartdridge, both the smartphone and the cartridge are assembled with the smartphone adapter creating a mini-dark box to perform measurement of the CL signal. Calibration curves were generated by adding known amounts of OTA standard solutions to wine and instant coffee OTA free founding a limit of detection of 0.3 μg L^{-1} in wine and 1.0 μg L^{-1} in instant coffee and dynamic ranges were respectively 0.3-50 μg L^{-1} and 1.0-56 μg L^{-1}. The developed system is suitable for screening procedures aimed at the quantitative detection of OTA in wine and instant coffee samples complying with EC legislation requirements.

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Development of a Chemiluminescent Immunoassay for Detecting ATP, as a Biomarker of Extant Life in Planetary Explorations

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Several studies reported the presence of organic compounds in extraterrestrial environments, thus one of the main objectives of the research in the field of exobiology is the search for organic molecules and compounds in planetary exploration missions and the identification of their biological or non-biological origin. Thus several research activities are dedicated to the development of devices capable of in-situ analyzing material samples in search of organic molecules, amino acids, nucleic acids, polysaccharides and other molecular systems characteristic of organized biological systems. An important example is given by the development of the Life Marker Chip (LMC)[1].

The increasing development of extremely compact systems relying on microfluidics, commonly known as lab-on-chip (LOC) devices, has gained much attention thanks to their favorable characteristics in terms of reduced size and weight, amenability to automation, very low sample and reagent consumption, reduced analysis time and, often, superior achievable performances in terms of limits-of-detection. Thus, LOC devices are extremely suitable for space missions and are under investigation in view of future planetary exploration.

Herein, we report about the development of a competitive immunoassay based on chemiluminescent (CL) detection for the identification of Adenosine triphosphate (ATP), which is commonly accepted as a high ranking biomarker of extant life in extraterrestrial environments. It is known that CL-based detection allows developing bioassays characterized by high detectability and sensitivity and it is also particularly suited for miniaturized analytical devices, as it avoids the need for external radiation sources and complex optical systems combining filters and lenses[2]. The immunoassay will be implemented into a portable device that is being developed exploiting a microfluidic network based on capillary forces for the handling of samples and reagents, integrated with an array of thin film hydrogenated amorphous silicon (a-Si:H) photosensors for the detection of the analytical chemiluminescent signal[3]. The implementation of the CL bioassay into the compact and fully-integrated device will provide a new analytical platform for the multiparametric detection of bio-organic molecules outside of the Earth.

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SOMMARIO – PROGRAMMA – [MEDAGLIE E PREMI](MEDAGLIE) – PLENARIE - KEYNOTE – ORALI- POSTER - AUTORI

Inorganic Components and Redox Behaviour as "Fingerprint" Of Italian Extravirgin Oil

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High quality olive oil, virgin and in particular extra virgin olive oil, are appreciated by consumers for both health benefits and pleasant flavour. Italy is by far the first country in Europe in terms of number of Protected Designation of Origin (PDO) oils, which usually relates consumers to a warm feeling of tradition, and thus to higher quality standards. Quality control issues are mainly related to a lack of sufficiently-powerful analytical methods, but also to a non objective classification of olive oil (OO), in particular related to the distinction between virgin oil (VO) and extra-virgin oil (EVOO). To support and reinforce the promotion, the valorisation of PDOs has also been suggested to increase the competitiveness of a country like Italy at international level. To modify such a scenario a strong scientific background is required, to emphasize all the different aspects related to high-added-value EVOO, such as quality, authenticity, and health benefits. This work is focused on target chemicals with high information valence in the direction of authentication, safety and product valorization by a detailed characterization of quali-quantitative inorganic profiling in EVOOs coming from different Italian regions[1]. The presence of metals in edible oils may be due to different factors: the metals can be incorporated into the oil from the soil or be introduced during the manufacturing of the foodstuff. Therefore, it can be assumed that the trace elemental distribution in olive oils varies according to their origin and then it can be supposed that a suitable statistical treatment on trace element data could allow a geographical characterization of different OOs. The total element content has been determined after pretreatment by acid digestion. An experimental design has been performed to optimize the conditions for the sample pretreatment, since a great variability was initially observed among the replicates for the same oil. When accepted value of relative standard deviations ($< 10\%$) were obtained, a certified material and ten EVOOs from different Italian regions were analysed. The concentration of the inorganic elements at trace and ultra-trace levels using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) has been determined[2]. Moreover, electrochemical properties of EVOOs have been investigated by voltammetry. For this purpose, carbon paste electrodes (CPE) were made mixing graphite powder and an aliquot of each sample; then, square-wave-voltammetry profile was registered for each CPE in a 0.1 M HCl solution. The features observed in the voltammograms reflect the reactions of electroactive compounds (such as polyphenols), which are present in the virgin olive oils mixed with the carbon paste matrix. For this reason, the voltammetric responses of the electrodes are specific for each type of oil[3]. Finally, chemometric treatments of the results were performed to assess the possibility to distinguish the region of provenience of each EVOO on the base of metal content and/or current registered during voltammetric analysis.

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Quantificazione Di Caffeina Ed Acido Clorogenico Nei Chicchi Di Caffè Verde Mediante HPLC-DAD E Chemiometria

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Una procedura cromatografica basata su un gradiente di separazione rapido è stata combinata con il metodo chemiometrico di deconvoluzione di segnali Multi Curve Resolution-Alternating Least Squares, (MCR-ALS)[1-3] al fine di quantificare la caffeina e l'acido clorogenico presenti nei chicchi di caffè verde. Sono state seguite due differenti procedure al fine di ottenere gli estratti da analizzare mediante HPLC-DAD; in entrambi i casi i chicchi sono stati polverizzati ed in seguito, l'estrazione ha avuto luogo mediante acqua o mediante miscela di solventi (metanolo/acqua). Dopo l'aggiunta dello standard interno (4-nitroanilina), gli estratti sono stati iniettati in HPLC (fase debole: acqua milli-Q acidificata con acido formico, fase forte: metanolo acidificato con acido formico allo 0.1%). Nonostante i tempi della corsa cromatografica non fossero sufficienti per una effettiva risoluzione dei picchi relativi alle sostanze presenti nei chicchi di caffè verde, la successiva analisi chemiometrica ha permesso la loro deconvoluzione e, di conseguenza, la creazione di una retta di calibrazione mediante la quale i due composti di interesse (caffeina ed acido clorogenico) sono stati quantificati. In una seconda parte dello studio, è stata valutata la possibilità di classificare, mediante Partial Least Squares Discriminant Analysis (PLS-DA)[4] e Soft Independent Modeling of Class Analogies (SIMCA)[5-6] i campioni sulla base della diversa cultivar dei chicchi (Arabica o Robusta). Utilizzando SIMCA come metodo di classificazione, i risultati migliori sono stati conseguiti sui dati ottenuti mediante estrazione con miscela di solventi, raggiungendo una sensitività ed una specificità del 100% per entrambe le classi. Costruendo i modelli di classificazione con PLS-DA, si è ottenuta una corretta classificazione del 100% indipendentemente dalla procedura utilizzata per l'estrazione. A scopo di esempio, in Figura 1 sono stati riportati i risultati relativi alla classificazione mediante PLS-DA (Figura 1a), e SIMCA (Figura 1b ed 1c) relativi ai campioni estratti mediante miscela di solventi.
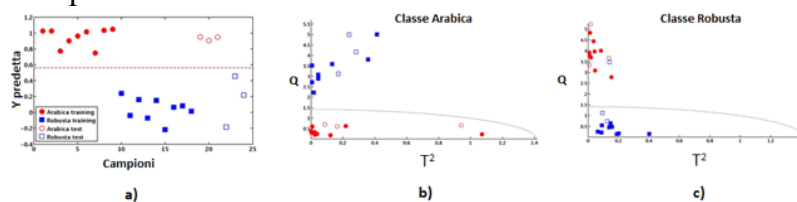
Figura: 3 Predizioni relative ai modelli: a) PLS-DA; b) SIMCA per la classe Arabica; c) SIMCA per la classe Robusta

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Caratterizzazione Di Ossa Fossili Provenienti Da Salme Sottoposte o Non A Cremazione Mediante Spettroscopia NIR, Analisi Termica e Chemiometria

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Dei reperti di ossa fossili (24 campioni) provenienti da quattro diverse necropoli, due in Italia (Cava degli Zucchi ed Elia-Velia) e due in Sudan (El Geili e Saggai) sono stati analizzati mediante analisi termica (termogravimetria) e spettroscopia NIR. Le misure termogravimetriche sono state effettuate sui campioni triturati, mentre l'analisi spettroscopica ha permesso di non modificare (e distruggere) i campioni. Le informazioni ottenute mediante le diverse tecniche sono state confrontate al fine di poter evidenziare e studiare eventuali differenze tra i reperti provenienti dalle diverse necropoli. L'interesse della comparazione risiede nel fatto che i campioni provenienti dall'Africa (non solo differiscono per l'origine), ma hanno subito anche una diversa sepoltura, che non prevedeva la cremazione dei corpi, che invece è stata praticata sui reperti provenienti dalle due necropoli site in Italia. I reperti risalgono ad epoche storiche differenti; romanica/preromanica quelle italiane, di epoca preistorica, meroitica e cristiana quelle sudanesi. Eventuali differenze/somiglianze all'interno del set di dati sono state investigate estraendo l'informazione presente in esso mediante l'Analisi delle Componenti Principali (PCA)[1-3]. A tal fine, i modelli PCA sono stati calcolati sulle matrici individuali (spettri NIR e termogrammi) ed in un'analisi congiunta (*multi-block*), calcolando il modello PCA sulla matrice dei *super-scores* ottenuta concatenando le componenti estratte dai modelli individuali. L'investigazione degli scores plot così ottenuti ha permesso di evidenziare alcuni raggruppamenti all'interno del data set. In particolare, la prima componente principale permette di distinguere i reperti sulla base del rito funebre praticato, infatti, i campioni cremati cadono a valori positivi della prima PC1 mentre gli altri a valori negativi. La seconda componente è connessa all'informazione relativa all'epoca a cui risalgono i reperti. Infatti, i campioni più antichi, cioè di tipo preistorico, si presentano a valori elevati di PC2, i campioni di epoca romana/preromana si trovano intorno allo zero della seconda componente principale ed i campioni cristiani cadono a valori negativi della PC2.

Figura 4 Scores Plot del modello PCA calcolato sui dati ottenuti mediante analisi termica. Legenda: in blu campioni cremati, in celeste campioni non cremati risalenti all'era preistorica, in verde e giallo campioni non cremati risalenti rispettivamente all'epoca meroitica e cristiana.
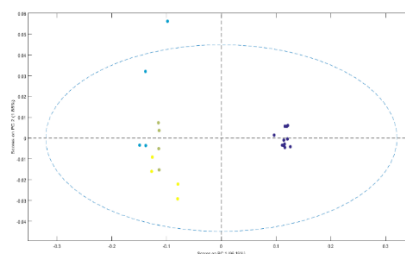
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Locally Weighted SO-PLS

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L'affinarsi delle moderne strumentazioni per l'analisi chimica ha permesso, soprattutto negli anni più recenti, che sempre più comunemente ci si trovi a dover analizzare i cosiddetti *multi-block data set*, ovvero, set di dati estratti dalle stesse matrici mediante diverse tecniche analitiche. I metodi di regressione multi-block sono stati sviluppati al fine di effettuare delle predizioni a partire da due o più blocchi di variabili indipendenti. In letteratura sono stati presentati diversi procedimenti per effettuare l'analisi dei dati mediante *data-fusion*; in diversi lavori è stata evidenziata l'efficacia delle metodologie multi-block rispetto all'analisi individuale di ogni singolo set di dati. Un altro effetto del progredire della tecnologia è la possibilità di ottenere dei dati sempre più complessi ed informativi. Questa caratteristica, che rappresenta indubbiamente un vantaggio, fa però sì che tutti gli effetti provenienti da diverse fonti di varianza, compresi effetti moltiplicativi indesiderati e noise strumentali non linearmente correlati con i fenomeni di interesse, siano più marcati. Di conseguenza, non sempre è possibile utilizzare dei metodi che siano basati su assunzioni di linearità all'interno dei dati. Nei casi in cui la non-linearità sia particolarmente evidente, è bene che i dati vengano analizzati mediante metodi sviluppati appositamente. In letteratura è possibile trovare diversi metodi elaborati al fine di risolvere problemi di dati non lineari in diversi ambiti, sia per l'analisi esplorativa che in regressione. Nonostante ci sia un notevole interesse sia per lo studio dei metodi non-lineari che per i metodi multi-block, in letteratura si trovano pochi esempi in cui questi due aspetti siano stati contestualmente investigati. Di conseguenza, lo scopo del presente lavoro è stato quello di sviluppare un metodo multi-block di regressione che permetta di analizzare dati chimici relativi a fenomeni non lineari. A tal fine, si è partiti da un metodo multi-block chiamato Sequential and Orthogonalized-PLS (SO-PLS)[1], e lo si è elaborato al fine di adattarlo all'analisi di modelli non lineari. Nello specifico, si è investigata la possibilità di creare dei modelli SO-PLS su dei sotto-gruppi dei campioni del data set, valutando localmente le distanze relative dei campioni sotto studio. Operativamente, la procedura proposta è di elaborare, inizialmente, un modello SO-PLS standard utilizzando tutti i dati presenti nel data set; in seguito, i campioni vengono proiettati nello spazio delle prime variabili latenti dei due blocchi predittivi e tutte le distanze euclidee tra i diversi campioni vengono calcolate. Di volta in volta, solo i campioni di training più vicini al campione incognito da predire sono utilizzati per la costruzione del modello multi-block; di conseguenza, ogni campione sarà predetto attraverso un differente modello locale. Sulla base di queste caratteristiche, il metodo proposto è stato chiamato locally-weighted-SO-PLS ed è stato esteso anche nell'ambito della classificazione, mediante combinazione con la Linear Discriminant Analysis (LDA)[2]. Il metodo è stato testato su diversi data sets, sia in regressione che in classificazione.

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Fourier Transform Infrared Spectroscopy and Termogravimetric Analysis in the Leather Quality Control: the Project LIFETAN (LIFE14 ENV/IT/000443)

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LIFETAN project is aimed at demonstrating the use of innovative natural products and technologies in the leather tanning process. The current commercial chemicals employed in the tanning process are very toxic and have a big impact on the environment. For this reason, EU supports Life+ Program in order to replace products/processes with eco-sustainable and convenient ones to propose "green" industrial cycles.

LIFETAN implements the results obtained in five previous Life+ projects that successfully demonstrated the use of natural products in the tanning cycle: OXATAN for the tanning phase, PODEBA for bating, ECODEFATTING for defatting, ECOFATTING for the fatting phase, BIONAD for the dyeing phase. LIFETAN as well as all Life+ projects demonstrate the use of the new natural products at laboratory, semi-industrial and industrial phase. The goals are to propose products with higher biodegradability and performance to produce high quality leather products better or comparable with those obtained employing traditional ones.

Spectroscopic techniques are fundamental in the monitoring actions to characterize the leather samples. In particular FTIR is a valuable useful technique to investigate at molecular level the interaction of new products with the leather proteins. The FT-IR analysis of amide I band gives information both in terms of the absorbance ratios at two different wavelengths (e.g. the 1654/1690 cm-1 absorbance ratio to evaluate the collagen cross linking) and the analysis of the single components found by peak fitting (conformational analysis). The results obtained on several LIFETAN samples obtained with the new tanning formulations are described and compared with thermogravimetric (TGA) data.

Transparent carbon nanotube network for efficient electrochemiluminescence device

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Electrochemiluminescence (ECL), or electrochemically generated chemiluminescence, is a leading technique in bioanalysis.(1) Since the excited species are produced with an electrochemical stimulus rather than with a light excitation source, ECL displays improved signal-to-noise ratio compared to photoluminescence, with minimized effects due to light scattering and luminescence background.(2) The peculiar analytical performances in terms of high detectability of conventional chemiluminescence (CL) are retained and, in addition, the electrochemical trigger of the reaction allows controlling the time and position of light emission from ECL probes. These properties make ECL systems particularly attractive also for microscopy imaging techniques in biological tissue sections or single cells, such as *in situ* hybridization (ISH) and immunohystochemistry (IHC).

In the quest for ever-increasing sensitivities, ECL can ideally be coupled to nanotechnology and supramolecular chemistry to develop new systems and strategies for analyte determination also in very complex matrices. For instance, we have recently shown a supramolecular approach to detect sarcosine, a potential prostate cancer biomarker, in urines, with good sensitivity and very high selectivity.(3) In this context, the nanostructured materials such as carbon nanotubes (CNTs) and graphene are particularly promising for sensing applications. (4)

Here we present the application of optically transparent electrodes based on carbon nanotubes and graphene based materials to ECL, (5) demonstrating the electrocatalytic superiority of such materials *vis-à-vis* ITO electrodes. CNTs and graphene electrodes are excellent material for ECL application thanks to the very favourable overpotential of amine oxidation that represents the rate-determining step for the signal generation in both research systems and commercial instrumentation. The employ of carbon nanotubes resulted in a ten times higher emission efficiency compared to commercial transparent ITO electrodes. Finally, we demonstrate as a proof of principle that our CNT device can be used for ECL imaging in which micro-beads were used to mimic a real biological sample, such as single cell visualization.
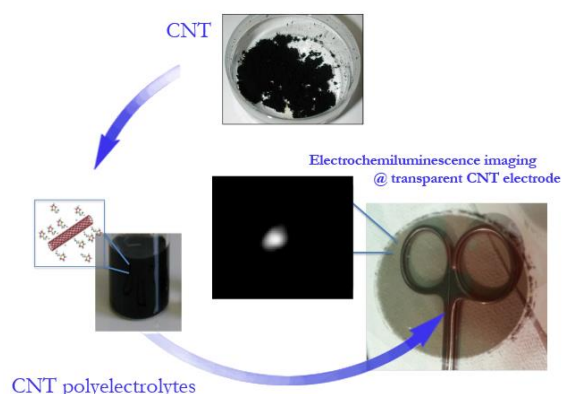
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Geographical discrimination of saffron (*Crocus sativus* L) by ICP-MS elemental data and class modelling of PDO *Zafferano dell'Aquila* produced in Abruzzo (Italy)

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Saffron, the spice obtained by drying of stigmas of *Crocus sativus* L., is the most expensive in the world and one of the most frequently adulterated foodstuffs. Therefore, certifying saffron origin and authenticity is of great economic importance, especially for the spices cultivated and entirely manufactured in given geographical areas following traditional methods, which add specificity and commercial value to the product making it competitive in the market. In the European Union, traditional specialties are valorized by means of specific regulations and marks such as the Protected Designation of Origin (PDO). Saffron cultivated near L'Aquila (Piana di Navelli), *Zafferano dell'Aquila*, is one of the five spices produced in Europe that can currently exhibit the PDO label. In the last decades, discrimination of saffron based on the geographical origin has been attempted by classification methods applied to experimental data obtained by both separative methods (HPLC and GC) and untargeted approaches (NMR, IR, UV-vis spectroscopy, among the others)[1,2]. In all the above approaches, discrimination is based on the difference in the metabolic profile, which is expected to be related with the production area and the local know-how applied in the post-harvest drying process. Trace mineral composition, on the other hand, was only rarely considered in the geographical traceability of saffron[3].

In this study, we further explore the potentiality of the elemental composition, determined by inductively coupled plasma-mass spectrometry (ICP-MS), in the geographical traceability of saffron. ICP-MS data of 52 elements collected on samples of *Zafferano dell'Aquila*, saffron samples produced in Iran, commercial products and spices cultivated in other Italian areas (Sardinia and Umbria) were preliminarily handled by unsupervised (principal component analysis) and supervised (linear discriminant analysis) multivariate methods. In addition, some class modelling techniques[4] were applied to evaluate the usefulness of the mineral composition in the authenticity verification of the PDO *Zafferano dell'Aquila*.

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"Spatio-spectral fuzzy-clustering" come vincolo spaziale per la risoluzione di immagini iperspettrali

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A differenza dei metodi di clustering tradizionali, nei quali ogni punto viene assegnato ad uno ed un solo gruppo, in maniera tale che tutti i punti in un cluster siano tra di loro i più simili possibile (e quanto più diversi possibile dai punti negli altri gruppi), gli algoritmi di fuzzy clustering prevedono che ogni osservazione possa appartenere a più di un cluster, associandole una probabilità per ciascuna delle attribuzioni. Per l'analisi delle immagini iperspettrali, ovvero delle immagini digitali per le quali a ciascun pixel sia associato un intero spettro, le tecniche di fuzzy clustering sono state in genere applicate considerando l'immagine come un insieme di spettri, ovvero senza prendere in considerazione l'informazione spaziale. D'altro canto, la possibilità di utilizzare l'informazione spaziale nell'ambito del fuzzy clustering è stata invece considerata nella risoluzione di immagini monocromatiche, come quelle ottenute dalla risonanza magnetica nucleare applicata nel campo del *medical imaging*[1]. Partendo da queste considerazioni, si è deciso di definire un algoritmo che permettesse di calcolare la funzione di appartenenza di ciascun pixel di un'immagine iperspettrale attraverso una procedura di fuzzy clustering che combinasse in sé l'informazione spaziale e spettrale (*spatio-spectral fuzzy clustering*). Questa procedura procede definendo una prima probabilità di appartenenza di ciascun pixel ai vari clusters sulla base della sola informazione spettrale, ed aggiornando successivamente questa probabilità sulla base della distribuzione spaziale delle funzioni di appartenenza: in questo modo, l'appartenenza di un pixel a un particolare cluster sarà tanto più probabile quanto maggiore sarà il numero di pixel vicini con alta probabilità di appartenere allo stesso gruppo. In questo contesto, obiettivo principale di questo lavoro è stato quello di verificare la possibilità di inserire l'algoritmo di *spatio-spectral fuzzy clustering* come vincolo all'interno dell'algoritmo iterativo *Multivariate Curve Resolution-Alternating Least Squares* (MCR-ALS)[2] al fine di migliorare la risoluzione spaziale delle immagini di tipo iperspettrale e rendere maggiormente evidenti ed omogenee le aree in cui le componenti di un campione siano distribuite. In particolare, si stabilisce di avere un numero di clusters uguale al numero di componenti nella risoluzione a cui applicare il vincolo e, ad ogni iterazione, si calcolano le funzioni di appartenenza per ciascun pixel sulla base della stima delle mappe di concentrazione. Queste funzioni di appartenenza vengono quindi utilizzate come pesi per calcolare una nuova stima delle mappe di concentrazione da usare nell'iterazione successiva dell'algoritmo ALS. Il procedimento viene ripetuto fino a convergenza dell'algoritmo stesso. Questo procedimento è stato applicato a due diversi campioni di emulsione, ossia una emulsione di olio di paraffina disperso in acqua ed un campione di maionese, preso ad esempio di emulsione commerciale. In entrambi i casi, l'algoritmo MCR è stato applicato utilizzando, oltre allo *spatio-spectral fuzzy clustering* oggetto di studio, il vincolo di non-negatività applicato ai profili spettrali e di concentrazione.

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Fluorescence spectroscopy and chemometric techniques for geographical discrimination of green tea samples

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The geographical origin of tea samples is an important factor to determine quality and reputation of a commercial tea product. Recently, different analytical procedures have been proposed for classification of different varieties or geographical origin of teas, including HPLC, elemental analysis, UV–vis spectroscopy, Near-Infrared spectroscopy, electronic tongue, electronic nose, multispectral or hyperspectral imaging, HPLC-DAD-MS. In our previous study[1], the content of catechins and methylxanthines in 92 green tea (GT) samples originating from China and Japan was determined by using micellar electrokinetic chromatography, and pattern recognition methods were applied to discriminate GT samples according to geographical origin.

In this study, an alternative analytical approach for the geographical discrimination of the GT samples is proposed, based on the combination of excitation–emission matrix (EEM) fluorescence spectroscopy as analytical technique and chemometric tools. Excitation-emission spectra were recorded using a Perkin–Elmer LS 55 (Perkin-Elmer Ltd., Beaconsfield, UK) luminescence spectrometer, with excitation and emission slits both set at 10 nm. Emission spectra were recorded between 295 and 800 nm, at excitation wavelength ranging from 200 to 290 nm. Principal Component Analysis was used as a display method, and considering the 3-way data sets (samples, excitation and emission wavelengths) PARAFAC[2] was applied as a multi-way decomposition method in order to extract the useful information from the spectra. The classification results obtained by EEM fluorescence spectroscopy were compared with those previously obtained by using micellar electrokinetic chromatography, finding a good agreement and thus demonstrating that fluorescence spectroscopy can be considered as a promising method for classifying GT by its origin.

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Effects Of Cellulose Oxidative And Hydrolytic Degradation Monitoring By Infrared Spectroscopy (ATR-FTIR) With Approach Of Principal Components Analysis

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In this study, we characterize the degradation phenomena of paper by inducing an artificial ageing on the standard paper (Whatman paper, tipe 1)[1] through chemical oxidation with potassium periodate, or acid hydrolysis with exposure to acid vapors hydrochloric acid and a mix of both treatments. The artificial cellulose degradation was investigated by FTIR-ATR techniques, pH analysis, HPLC analysis and FE-SEM measurements. Finally, principal components analysis (PCA) was used to analyze the data set of the results. All performed treatments, significantly change the structure and properties of the paper; at the same time results show that the extent of deterioration depends on the kind of ageing. Furthermore, cleaning gels will be applied on aged paper samples in order to test their applicability and cleaning capability on differently aged paper artworks.

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Evaluation of penetration depth for a NIR hyperspectral system at different wavelengths and on different matrices

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Hyperspectral imaging is usually considered as a surface analysis method. Nevertheless, electromagnetic rays may penetrate in depth, inside the upper layers of the sample, including information from the inner parts in the recorded spectra. Moreover, such an effect depends on the matrices analysed and on the specific wavelengths at which spectra are recorded.

The aim of this work was the evaluation of penetration depth for a near infrared hyperspectral imaging system working in the 1000–2500 nm spectral range, at 8 nm spectral resolution (SWIR3 camera equipped with a LabScanner 40x20).

Penetration depth was evaluated on several matrices characterised by different physico-chemical features, including chemical composition, colour, humidity, and microstructure.

To the aim, a chessboard background was assembled using two polymeric materials characterised by different total reflectance values (about 0% and 90%, respectively) and with different and characteristic spectral profiles. The use of a geometrical pattern, in comparison with uniform backgrounds, adds spatial information to the penetration depth evaluation. Images were recorded placing the matrices, both sliced at different thickness and wedge-shaped, between the source and the chessboard background.

Multivariate image analysis was carried out in order to highlight both spatial and spectral features, allowing to evaluate the penetration depth. First of all, a region of interest (ROI) within the hyperspectral image, including a representative portion of the sample was selected. Then, principal component analysis (PCA) was carried out, reducing data dimensionality and removing non-useful information to confirm the level of penetration on the basis of score images.

Up to now, hyperspectral imaging has been considered basically as a reflectance method but, on the basis of these outcomes, it should be regarded as a transflectance approach in many cases, depending, in particular, on the physico-chemical characteristics of the matrix, such as the chemical composition and the physical microstructure.

Quick Determination of Olive Oil Acidity by Voltammetry and Partial Least Square Regression

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Olive oil acidity is a key parameter routinely determined to classify and/or assess quality, freshness and economic value of the final commercial product. In a recent work[1], a novel electroanalytical approach was presented for the direct determination of acidity degree in different commercial categories of oils (extra-virgin, olive oil, lampante). Acidity levels of samples were obtained by exploiting the reduction process of oleic acid (OA), recorded by voltammetry at a platinum microelectrode, which allowed minimising most of the unwanted effects on the current-potential responses, which are typically encountered in viscous and low conducting media. Suitable conductivity of the samples was achieved by addition to oils the ionic liquid tri-hexyl(tetradecyl)phosphonium bis (trifluoromethylsulfonyl) imide $[P_{14,6,6,6}]^+[NTf_2]^-$, which acted as a supporting electrolyte. With the aim of increasing repeatability and accuracy of the voltammetric approach for the quantification of the acidity degree in extra-virgin olive oils, in which the acidity levels are very low, in the present work the application of a suited multivariate regression analysis to be coupled with the voltammetric responses was investigated.

In particular, several Italian extra virgin olive oils were sampled and measured under air-saturated conditions, both at their natural acidity content and at increasing acidity levels, obtained by adding different amounts of pure oleic acid (OA) – the main fatty acid in olive oil. Cyclic voltammetry (CV) at low scan rates was used as waveform for recording current against potential responses. Simultaneously, free fatty acid content (% FFA) was determined in the same samples using the official reference titration method.

After choosing the most suitable pre-processing for CV data, partial least squares (PLS) regression was applied on selected regions of the CV signals, as an alternative approach to the classical univariate regression. PLS allows to consider also the shape of entire current profiles and to obtain a quantitative relationship with the acidity content of samples. Models were optimised choosing the optimal complexity, in terms of latent variable (LV) number and root mean squared error in prediction (RMSEP). Validation was performed on a separate sample set.

Results suggest that the proposed procedure could be advantageously exploited for a quick, low-cost and solvent-saving analytical determinations of olive oil free acidity, employing very small sample volumes (<1 mL).

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XPS characterization of (Mo/Se) films grown by electrochemical atomic layer deposition

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The interest towards 2D materials for photovoltaic applications has grown dramatically in the last years[1]. In this field, metal dichalcogenides (MX_2) are widely studied owing to their peculiar optoelectronic properties[2]. An extremely versatile approach to develop layered MX_2 is represented by electrochemical atomic layer deposition (E-ALD), which allows the growth of thin films at ambient pressure and room temperature[3]. Recently, research about the preparation of $MoSe_2$ films by alternating the electrochemical deposition of Se and Mo onto Ag(111) substrates has been started in collaboration with University of Florence. A powerful tool for the characterization of similar materials is represented by X-ray photoelectron spectroscopy (XPS), as demonstrated in our previous works combining also morphological and (*in operando*) spectroscopic investigations[3-5]. In this communication, XPS analysis of Mo/Se multilayers is presented with a particular focus on element speciation and the influence of electrochemical deposition parameters on the final surface chemical composition of thin films.

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Mitochondrial proteome modifications due to eIF6 depletion by UHPLC-QTOF MS/MS with SWATH-MS acquisition

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Eukaryotic Initiation Factor 6 (eIF6) is an initiation factor that binds 60S ribosomal subunits and has an anti-association property, by hampering 60S premature joining to 40S. In general, eIF6 is rate limiting for tumour onset and progression. eIF6 haploinsufficient cells are normal, but not efficiently transformed in vitro. Mitochondria are the main compartments of energy production, and some lines of evidence have shown that mitochondrial alterations contribute to the development of metabolic syndrome.

To this aim, we analysed, by uHPLC-QTOF-MS/MS exploiting the SWATH-MS (Sequential Window Acquisition of all Theoretical fragment ion spectra) acquisition, the expression of mitochondrial proteome of AML-12 (non-tumourigenic murine liver hepatocytes) cell line, where eIF6 was down-regulated by shRNA, and of three different tissues from wild type and +/- mice for eIF6 (liver, muscle and brain).

The SWATH-MS acquisition method is a high throughput label-free method for protein quantitation that combines the traditional shotgun proteomics with the quantitative accuracy and reproducibility of selected reaction monitoring (SRM).

We found that depletion of eIF6 by shRNA induces profound and varied impact on mitochondrial proteome, impairing the energy production, steering the metabolism toward the up-regulation of aerobic glycolysis and the inhibition of oxidative phosphorylation.

Authentication Study Along The Production Chain Of Hazelnut Paste By ICP-MS And Multivariate Analysis

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Hazelnuts are widespread in the food industry either as whole fruits, small grains, flour or oil, to produce paste, cream and other finished products, above all in confectionery. Different hazelnut cultivars show a quite different commercial value, the cultivar "Tonda gentile delle Langhe" produced in Italy being by far the most valuable. Along the industrial production chain, hazelnuts first undergo a process of roasting to exalt their aroma, and then hazelnut paste is produced by a fine grinding of the peeled fruits.

Due to the great commercial difference between the "Tonda gentile delle Langhe" and the other cultivars, it is particularly important for the food industry the availability of tools for tracing the variety and the geographic provenance of hazelnuts and their derivatives along the production chain of the hazelnut paste.

Here, we apply elemental profiling through ICP-MS and ICP-OES in an authentication study involving the production chain of hazelnut paste: from raw fruits, to roasted hazelnuts, down to the final paste. The study involves three different cultivars ("Tonda Gentile delle Langhe", "Romana" and "Mortarella"). PLS-DA with variable selection was applied to identify a model able to distinguish the three cultivars, independently on the type of technological transformation, and to search for elemental markers of authenticity. The model performances were very good both in fitting and cross-validation and proved the applicability of the procedure for the authentication of hazelnuts along the production chain of hazelnut paste. To the authors knowledge, this is the first authentication study involving the production chain of this product.

Rare Earth Elements Analysis For Extra Virgin Olive Oil Assessment: The Case Study Of Tuscany Olive Groves, Italy

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Traceability of the food origin is important for label protection. Food fraud is a growing problem that costs the *global* food industry between \$10 billion and \$15 billion per year. Food ingredient fraud and economically ingredient adulteration are emerging risks, but a comprehensive compilation of information about known problematic ingredients and detection methods does not currently exist. The olive oil is in the E.U. top ten list of food at the most risk of fraud. Chemical analyses per se are not sufficient to verify olive oil authenticity except in cases of adulteration with other vegetables oil. Until now, the analytical criteria available for the international regulations on the definition of oil genuineness and quality are based on the determination of classic parameters: acidity, peroxide value, ultraviolet absorption and the fatty acid composition. The above mentioned analyses are not able to trace the origin of the olive oil.

This research project, called "GEOEVO", started in 2016 and proposes an alternative method to evaluate the geographic origin of olive oils by determining the rare earth elements (REEs) within the soil and corresponding olive oils[1]. Starting from the assumption that the REEs present in the soils and substrates are uptaken by plant roots. We hypothesize that different geological substrates give a combination of REEs that characterize and influence the quality and traceability of the olive oil[2]. This study will focus on the following lithologies: (i) volcanic rocks, (ii) sandstones (iii) and limestones. The specific objectives are: (i) to find a response in the absorption spectra of rare earths in soil and in olive oil samples taken as a function of different lithologies; (ii) to identify mixtures of different olive oils (blend); (iii) to calculate a statistical probability distribution of REEs in soil and in corresponding olive oil. REEs concentration results will be analyzed by statistic techniques, ANN and PCA, to reveal if the data obtained are suitable to discriminate between olive oils from different geographical origin. Sampling of soils, olives and olive oils was carried out in ten olive groves located in Tuscany. First results were reported and discussed.

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Rapid Quantification Of Biosilica In Marine Sediments By ATR And Net Analyte Signal

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A novel chemometric approach based on Net Analyte Signal (NAS) has been developed and applied to the quantification of biosilica in marine sediments.

The official method for the determination of this biogenic marker is based on a wet method[1], which involves the dissolution of the sediment in a strong alkaline environment and subsequent spectrophotometric assessment. Therefore, it is a destructive method, seriously affecting precision and accuracy of analysis; moreover, the mineralization process is matrix-dependent, involving the need for different conditions to be set-up according to the different sediment investigated.

In this work in order to overcome the troublesome traditional approach, we propose a non-destructive method for the assessment of biosilica based on ATR spectroscopy and the use standard addition approach. Sediments aliquots are desiccated at 105°C, sieved and grinded with a ball mill. Then they are split in four aliquots: the first one is the zero-added sample, while a growing amounts of pure celite as a proxy for bio-silica is added to the others. These powder samples are then directly analysed by Infrared Spectroscopy in ATR mode (ATR). ATR has the great advantages of being a rapid and non-destructive technique, requiring a little amount of sample, while the complexity of the spectra requires a smart chemometric approach to solve for quantitative information they contain in implicit form.

IR spectra are therefore elaborated by the Net Analyte Signal (NAS) algorithm[2,3] which allows to extract the contribution of the analyte from the whole signal. In this way, the problem of the matrix effect affecting spectra is solved. The Euclidean norm of the NAS signal is then used to create a pseudo-univariate standard addition model, from which the concentration of biosilica in the starting sediment is extrapolated.

In order to validate the IR method, three sediment samples collected in the Station D in Ross Sea, Antarctic, at different depth have been analysed, using both the wet method and the ATR-NAS method. Results of the two methods are in good agreement, showing that NAS can be used to successfully quantify biosilica in marine sediments using a a faster, cheaper and more universal method than the traditional one.

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The Role of Organic Gunshot Residues In Criminal Investigation

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This work has been focused on the developement of analytical protocols able to find solutions to the critical issues of current methods in gunshot residues investigation. In particular, a method based on High Performance Liquid Chromatography hyphenated to Mass Spectrometry (HPLC-MS/MS) was considered to analyze the organic components of the gunshot residues (OGSR). In fact, sensitive and robust analytical technique is essential to face an increasing number of incidents related to firearms.

Several approaches have been considered for the collection of the commonly determined primer-metal residues from the hands and clothing including swabbing[1], lifting with an adhesive tape or stub[2], and vacuuming[3], but they did not solve critical issues related to these components that may be very challenging in such situations involving lead-less cartridge.

In order to optimize the analytic method, shooting experiments were performed to evaluate transfer of OGSR using different cartdriges fired by the same shotguns, so as to find features particles of the ammunition used.

Using LC–MS in positive electrospray ionization (ESI) mode, eleven major stabilizers found in OGSR were targeted, while three compounds in negative ionization were characterized.

The achieved results have shown that, using the results derived from the analysis of OGSR, we could be draw information for investigative purposes, to give confirmation of the hypothesis of crime, making more accurate the interpretation of the analytical results.

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Forensic Examination of Black Toners In Questioned Documents By NIR Spectroscopy And Chemometrics

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The examination of questioned documents represents a fundamental step in forensic investigation as it provides information about the origin and similarities of a document to detect potential falsifications[1]. In particular, the analysis of printing toners with the aim of discriminating brands, manufactures and printers involved, is becoming very challenging as the use of office and personal laser printers and photocopying machines dramatically increased[2].

In this work, the feasibility of NIR/Chemometrics was investigated as a novel way to analyse black toners in questioned documents. Ten black toners from four manufacturers were included in this study and the acquired NIR spectra were compared in order to differentiate toners. Multivariate statistical analysis based on Principal Component Analysis (PCA) was considered to optimise the model for the identification of toners in questioned documents.

Results demonstrated the ability of NIR spectroscopy to simultaneously differentiate toners according to the brand and also to a typical composition among toners of the same manufacture. Moreover, the method proved to be not affected neither by the type of paper involved, nor by the way documents are printed suggesting to be a very promising tool to compare documents of unknown origin and allowing discrimination between them. The good experimental results show the opportunity of considering the proposed approach into procedures for forensic documents examination.

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Validation of an Analytical Method for the Determination Illicit Drugs in Hair Based on LC-MS/MS and Comparison of Different Extraction Methods

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In the last twenty years, hair testing has gained increasing attention and recognition as a complement to blood and urine analysis. Hair differs from other materials used for toxicological analysis, due to its large detection window which is weeks to months depending[1]. Other advantages are the easy sample collection, storage and transport. Moreover, segmental analysis of hair strands allows the determination of the historic pattern of drug use. Consequently, hair analysis of illicit drugs and medicines is currently employed to address a wide range of challenges, such as drug abuse history, workplace testing, post-mortem toxicology, therapeutic drug monitoring or drug facilitated assault (DFA) investigations. The most crucial issue facing hair analysis is the avoidance of false-positive results caused by passive exposure to the drug. For this reason, SoHT recommends that hair analysis starts with a wash step to remove external contamination[2]. The extraction of psycoactive substances from the inner of the hair is a critical point of the analytical process: different methods have been proposed[3], but most of them include a limited number of analyte. For hair incubation, the most used method is the digestion of hair matrix with NOH, alternative methods consist in the incubation of hair with methanol or ethanol for several hours, up to 18. Recently we proposed a new method[4] that uses the pressurized liquid extraction (PLE), capable to obtain good recoveries in a short time. The clean-up of the sample obtained from the PLE were carried on by dispersive-liquid liquid microexcraction (dLLME). The aim of this work is to compare our method, based to PLE-dLLME, followed by HPLC-ESI-MS / MS, with classic ones considering retention times, matrix effect and recoveries. Some samples were fortified by adding an analytical solution to the hair, as is generally done in literature (spiked method), while a fortification method was then evaluated by "soaking" capable of simulating real specimens, as analytes are introduced into the hair[5]. Finally, the methods were also compared using real samples obtained from volunteer. In this work, we carried out the determination of 21 analytes, belonging to most classes of substance abuse between classics and new drugs. The results showed that the spiked fortification is unable to simulate a real sample. The proposed method is capable of obtaining a hair extraction in a few minutes, and the validation demonstrated the suitability on real samples and a clean and less influenced sample compared to other literature methods.

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Chemometric Approaches for the Interpretation of Direct Biomarkers of Ethanol Consumption for Forensic Purposes

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The quantitative determination of direct ethanol metabolites – i.e. ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEEs) – in hair samples represents the optimal analytical approach to identify chronic alcohol abusers. Cut-off values have been established by the Society of Hair Testing (SoHT) to interpret EtG and FAEEs results, but several confounding factors (e.g. cosmetic treatments, intra-individual variability, etc.) may alter the biomarkers' concentration in hair and their correlation with alcohol consumption, potentially leading to cut-off value crossing. As a consequence, the adoption of the traditional univariate interpretation approach is exposed to misclassification errors and misleading conclusions from forensic experts and physicians. The alternative use of multivariate data interpretation approaches is tested to infer more robust conclusions. Several likelihood ratio (LR) models were developed and validated to overcome the drawbacks of the traditional univariate approaches, that involve cut-off values in an oversimplified process of evidence evaluation. In contrast, LR approaches evaluates the evidence (E) in case of two different, and mutually exclusive, hypotheses by examining the collected data. The resulting LR values reveal the support to be delivered to the evaluated propositions and LR results can be expressed by means of verbal scales. In the present case, the first hypothesis (H1) is that the individual under examination is not a chronic alcohol consumer, while the second hypothesis (H2) states that the examined subject is a chronic alcohol misuser. The collected data include direct biomarkers of alcohol consumption (FAEEs, EtG) from more than 150 scalp hair samples of different individuals, representing both a-priori chronic and non-chronic alcohol drinkers targeted categories. The different multivariate LR models were compared and their ability to discriminate between chronic alcohol misusers from non-chronic alcohol consumers was examined. The performances of each model were evaluated in terms of correct classification rate (%) and empirical cross entropy parameters. Satisfactory reduction of information loss was finally observed, together with correct classification rates close to 100%, proving that LR validated models are able to discriminate non-chronic from chronic alcohol consumers. Similar efficiency was observed employing further multivariate data analysis strategies such as Partial Least Squares – Discriminant Analysis (PLS-DA). In conclusion, multivariate statistical approaches to the interpretation of alcohol abuse biomarkers seem to efficiently corroborate clinical evidences in the diagnoses of chronic abuse.

Chemometric Interpretation Of GC-MS Fire Debris Results

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In the sphere of fire debris investigations, the analysis of collected evidences represents the main tool to recognize the occurrence of an alleged arson. The results from GC-MS analysis performed on collected fire debris may be compared to those obtained from ignitable liquids. Different gasolines were sampled from different oil stations located within the area of the city of Turin and then analysed with the aim of evaluating the chance to effectively discriminate their different origin. The study was conducted within the collaboration between the Department of Chemistry and the Turin Fire Department. The use of various approaches of multivariate data analysis allowed us to build classification and likelihood ratio models indicating the probabilities that specific fire accelerants have been employed in real arson casework. In particular, both fresh and weathered samples at different percentages (i.e. 25%, 50%, 75%, 95%) were analysed and compared to standard mixtures (ASTM1618). Furthermore, a data set including more than 150 GC-MS analysis relative to 30 oil stations was used for multivariate analysis. The collected mass-chromatograms were, in fact, interpreted using a variety of targeted and untargeted approaches of multivariate data analysis, using both raw and semi-quantitative data. In particular, Principal Component Analysis (PCA) and N-Way strategies such as Tucker3 and Parallel Factor Analysis (PARAFAC) were employed with the goal of recognizing and discriminating the usage of different fire accelerants. More in details, Principal Component Analysis allowed to locate the fresh gasoline samples within a scores plot according to their origin, i.e. the different oil stations. Moreover, N-Way strategies were tested in order to figure out how multivariate strategies were able to assess the occurrence of fire accelerant in an arson scene. To this purpose, whole tridimensional GC-MS data collected in scan mode were compared with those obtained from different oil stations gasoline samples. Then, likelihood ratio approaches have been evaluated for the same purpose, by evaluating samples that were burnt in presence or in absence of fire accelerants with the integration of Bayesian's logic. Further development of multivariate feature-based and score-based likelihood ratio models built on the collected GC-MS spectra are in progress, too.

Mechanistic insights into the ultrasensitivie determination of iron by adsorptive voltammetry with catalytic enhancement

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The detection of iron and, subsequently, iron speciation analysis made their way into the forefront issues in chemical oceanography following the discovery that iron limits algal growth (primary productivity) in substantial areas of the oceans (first formulation of the iron hypothesis in 1990 (1)). Far fetching consequences on the carbon dioxide global cycle and, consequently, on climate change have been rapidly acknowledged (1). Nevertheless, iron detection in seawater demonstrated among the most challenging topics in inorganic analytical chemistry due to the poor solubility (picomolar to nanomolar) of iron in oxygen saturated alkaline waters.

Electrochemical techniques had already emerged since mid-Seventies as reliable methods to determine a number of trace elements in solution, employing in situ accumulation techniques to achieve the required low limits of detection. Procedures based on anodic stripping voltammetry (ASV) were firstly developed followed by methods based on cathodic stripping voltammetry (AdCSV, adsorptive cathodic stripping voltammetry). They efficiently faced the issues posed by the saline matrix and the extremely low concentrations although required of a digestion step to cancel interferences from natural organic matter. The first method for iron detection in seawater by AdCSV was introduced in 1984 by van den Berg et al. (2). A substantial increase in performance was achieved in the last five years by introducing noteworthy, sequential improvements to previously developed methods. The procedure employing 2,3-dihydroxynaphthalene (DHN) and an oxidiser to enhance sensitivity, proposed in 2001 by Obata and van den Berg (3), was mainly pursued. Firstly, an increase in sample pH to 8.7 lead to a ~threefold decrease in the LOD (4): the same study demonstrated that this procedure does not require UV digestion prior to analysis at low organic matter concentrations, albeit an overnight acidification of the sample is necessary. In the following year, oxygen was introduced as a sensitivity enhancer for the detection of the Fe-(salycilaldoxime)$_2$ complex, presumably acting as a precursor for hydrogen peroxide (5), although the method was developed for speciation analysis and shows limited sensitivity. Dioxygen as a signal enhancer was subsequently applied to the methodology employing DHN as the complexing ligand and a small volume cell requiring 500 µL of sample only. The latter procedure achieved a LOD of 5 pM employing a deposition time of 30 s, reducing the overall analysis time to 8 minutes per sample (6).

Such a great improvement in detection capabilities and in the overall procedure was not paralleled by an understanding of the involved chemical processesse. Aim of the present communication is to underpin the mechanism responsible for such a high sensitivity and to establish a framework for the assessment of AdCSV procedures. These data will be employed to try and define alternative ligands or procedures which may provide better performances not only for total iron detection but also for the speciation procedure.

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Multiple Reaction Monitoring tandem mass spectrometry for the selective quantitative determination of Oxylipins in serum from n patients with systemic sclerosis

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Oxylipins are a new class of oxygenated metabolites of polyunsaturated fatty acids (PUFAs) that affect a broad range of physiological processes, including interestingly the initiation and signalling of the inflammatory response (1). The evident complexity of the cascade reactions triggered by inflammatory processes and the discovery of new oxylipin families with additional anti-inflammatory and pro-resolution effects has encourage researcher to set up analytical methods analysing a number of molecules as high as possible. Although the most of studies provide the isolation and analysis of single oxylipin species, only a limited number of analytical methods offer a comprehensive coverage of the oxylipin synthetic cascade applicable to a wide range of human biofluids. Nowadays, automatic rapid high performance liquid chromatography tandem mass spectrometry has become the technique of choice for several metabolite analysis thanks to the capacity to simultaneously separate and determinate multi component mixtures (2). Here, a method using liquid chromatography-electrospray tandem mass spectrometry (LC-MS/MS) in multiple reaction monitoring ion mode operating in negative ion mode has been developed for the determination of oxylipins pattern in serum from patients affected from systemic sclerosis. The simultaneous, sensitive, fast, and reproducible measurement of 80 oxylipins in a single MRM run might be useful in the clustering the pathology at different level of inflammation.

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Quantitative analysis of catechins by Multiple Reaction Monitoring in tea, coffee and food supplements.

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Catechins are secondary metabolites of plants, The main role of phenolic compounds as metabolites in plants is food deterrence; because of their bitter taste they play a role in defense, but not only, in fact: they give mechanical support, attract pollinator insects, absorb harmful UV rays and are substances allelopathic.

The contribution of polyphenols to human diet varies enormously in relation to the type, quantity and quality of the consumed vegetables; The intake is generally considerable, just because they are the most antioxidant molecules represented in the vegetable kingdom. The main role of catechins in humans is to protect cells from damage caused by free radicals that develop with normal cell metabolism due to stress events (1).

The aim of the work was to develop a tandem mass spectrometry strategy in Multiple Reaction Monitoring to identify and quantify the catechins mainly contained in commonly used drinks such as tea and coffee and the subsequent application of the optimized method for dietary supplements analysis present on the market. Their content in these drinks is variable and depends on plant variety, growth conditions, long cultivation, harvesting methods and times, as well as conservation and machining processes (2).

Therefore, 75 species were selected to be monitored during a single LC MRM MS (5 minutes) analysis and for each compound the best two precursor ion-fragment ions transitions were selected (thanks to literature data) to ensure high selectivity and sensitivity to the analysis

The quantitative analysis was performed using the external standard method in a range of concentrations ranging from 0.1 to 25 pg/L.

Analytical parameters (e.g. LOD, LOQ, linearity, intraday ripetitivity...) were determined.

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Nanoporous Graphene Oxide Assemblies on Electrodes for Electroanalytical Applications

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The knowledge to design and construct porous functional materials with tunable chemical and physical properties has recently attracted a lot of attention for chemical sensing applications. On this realm the unique properties of graphene offer unprecedented opportunities to development novel materials. This is further enhanced by the fact that the graphene-based materials can be covalently or non-covalently modified with a variety of functional groups on the surface, as it unlocks the potential to impart selective sensing capabilities. Here we are challenging the assembly of porous structures based on graphene oxide (GO) for the modification of electrode surfaces for electroanalytical applications. 3D nanoporous architectures were prepared by assembling 2D graphene oxide layers by using tetrapod-shaped linker molecules that act like pillars between the GO layers (1). The resulting porous structure is characterized by an expansion of inter-layer distance of GO from ~7.5Å to 13-14Å, which allows the intercalation of specific molecules (analyte) between the layers. The widths, volumes, and chemical properties of the pores of such structure can be tuned depending on the density of the pillaring molecules. The resulting materials were characterized for the degree of functionalization, GO sheets spacing, number of layers per flake and porosity. The assembled 3D structure was first tested for the adsorption of divalent metal ions (copper, zinc, cadmium and lead) from aqueous solutions and a model of the interaction between the metal ions and the oxygen containing groups of porous GO was proposed. These adsorption properties make the porous GO material of interest for the modification of electrode surfaces for the sensitive electrochemical analysis of heavy metals in water. Nanoporous GO-modified electrodes were prepared and their performances for the simultaneous detection of metals were tested via anodic stripping voltammetry.

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Volatile fraction analysis by HS-SPME/GC-MS and chemometric tools to characterize the flavor of *lager* craft beers

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Beer is one of the most popular and oldest alcoholic beverages in the world (1). In particular, *lager pilsner* is the category of low fermentation beers most common in continental Europe. In Italy, the production capacity of the breweries has increased along with the new ways of consumption: 13 million hectoliters of beer were produced in 2014, of which 3.3% of the production were handicrafts. Of course, this represents only a small part of the total but can already be considered surprising if one considers that in 2011 were only 1.1% (2).

Commercial beers are always microfiltrate and pasteurized while craft beers do not, and in fact the concept of artisanal beer among consumers is related to the highest quality beer. In Italy, however, the recent law does not mention the quality of the raw materials used, but defines only the production processes (3).

This study intends to characterize the two types of beer through the analysis of specific markers determined by studying the aromatic profile. The aromatic component of beer is very important, considering that aroma has a great influence on the consumer's acceptance and preferences of different products.

The headspace solid-phase microextraction (HS-SPME) technique followed by GC/MS analysis was performed to evaluate the volatile fraction of beer samples produced under different processes (handicraft methods or industrial processes). For headspace SPME was used a DVB/CAR/PDMS fiber who provided extraction of a wide range of volatile compounds with different polarities (4,5). The monitoring of volatile fraction, which contributes to flavor definition, may represent an interesting tool to characterize beer samples in relation to the manufacturing processes.

Principal Component Analysis (PCA) was applied to experimental data set to identify volatile compounds, who confer a particular aroma, suitable to be used as potential quality/process markers in order to discriminate beer samples according to their production method. Hence, the proposed method may help to protect the craft beer from label frauds by verifying whether samples comply with statements concerning conditions set out by the law.

The preliminary outcomes are promising and the study assesses the possibility of differentiating and enhancing product types on the market at different prices, and providing process/product indicators as a tool to distinguish a beer also on aspects defined as "added values" that would justify the adoption of a "premium price" for craft beers.

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Setup of new metrics in performance assessment and validation of NMR methods for fingerprinting and simultaneous multicomponent quantitative analysis

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The goal of this work was to set up validation procedures and new quality control parameters suitable for performance assessment in simultaneous multi component quantitative NMR analysis and NMR fingerprinting methods. In order to achieve the goal, three inter-laboratory comparisons (ILCs) were organized. The first one(1,2) consisted in the analysis of wheat and flours aqueous extracts (4 samples) and was aimed to ascertain the statistical equivalence of the scaled NMR spectra. 780 NMR spectra were produced by 32 participants using 39 different NMR spectrometers. Seven signals were submitted to univariate internationally agreed statistics typically applied in performance assessment of ILC participants.The second ILC(3) regarded a model mixture made up of five compounds. In particular, a model mixture made up of five compounds [Aldicarb, Methamidophos, Oxadixyl, Pirimicarb and 3-(trimethylsilyl)-2,2,3,3-tetradeutero-propionic acid sodium salt (TSP)] dissolved in deuterated water was submitted to NMR analyses. 1260 NMR spectra were produced by 30 participants using 34 different NMR spectrometers. The analytical target of the second ILC was the quantification of analytes by the calibration line method. Such a method was chosen as it allows for identification of a theoretical line to be taken as reference in performance assessment. The third ILC consisted in the analysis of wine grape juice (7 samples) and was aimed to confirm the statistical equivalence of the scaled NMR spectra and to quantify several metabolites by standard addition method. 2310 NMR spectra were produced by 54 participants using 66 different NMR spectrometers. Results show that quantitative NMR is a robust quantification tool. Performance assessment was carried out on single component quantification, by the popular and traditional z-score, and on multi-component analyses by means of a new performance index (named Q_p-score) which is related to the difference between the experimental and the consensus values of the slope of the calibration lines. By an analogous reasoning followed for z-score, performance assessment by Q_p-score is considered satisfactory when $|Q_p| \leq 2.0$, questionable when $2.0 < |Q_p| < 3.0$ and unsatisfactory when $|Q_p| \geq 3.0$. Qp-score is a parameter suitable for harmonization of fingerprinting protocols and simultaneous quantitative multi component analysis. Such parameter, that was designed considering consolidated internationally agreed statistics, represents an unbiased evaluation tools for NMR method validations.

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The oxidative potential of $PM_{2.5}$ and PM_{10} at an urban site in Lecce: influence of the sources of combustion and transport of Saharan dust

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Atmospheric particulate matter (PM) has adverse effects on human health (1). Although the PM's toxic effect on human health can be related to its chemical-physical properties, toxicity mechanisms are not yet fully known. Related studies suggested that reactive oxygen species (ROS) either transported on particles or catalytically generated by particles through red-ox reactions can be important sources of cellular damages. For this reason, in the international scientific community, the oxidative potential (OP) of atmospheric particulate matter is considered a practical indicator to investigate potential risks for specific human health problems.

Contribution to the oxidative potential of specific anthropogenic sources, such as road traffic, biomass combustion and industrial emissions, has been studied at several observation sites. However, at the present information regarding the OP of natural source are still insufficient. As for example, data related to the contribution of the Saharans dust (Saharan Dust Outbreak, SDO) on the oxidative potential are not yet available.

The aim of this work was to fill this gap by evaluating the OP of water-soluble $PM_{2.5}$ and PM_{10} samples taken at the Environmental-Climate Observatory of Lecce (Southern Italy, 40°20'8''N-18°07'28''E, 37 m asl), regional station of the Global Atmosphere Watch (GAW) network, by using the a-cellular DTT (dithiothreitol) assay (2). The OP values of three groups of samples were compared: standard having concentrations and carbon content similar to the yearly averages values; high carbon samples associated to combustion sources (mainly road traffic and biomass burning) and Saharan dust outbreaks (SDO). DTT activity normalised by volume (DTT_V), representative of personal exposure, and normalised by mass (DTT_M), representing source-specific characteristics, were investigated. The DTT_V is an extensive property that increases with the increase of PM concentration, well correlated with secondary organic carbon concentration. It is significantly larger in fine particles $PM_{2.5}$ with respect to coarse fraction $PM_{2.5-10}$. DTT_V is larger for high carbon content samples but during SDO events is statistically comparable with that of standard samples. DTT_M is larger on $PM_{2.5}$ with respect to PM_{10} and the relative differences between the two size fractions is maximised during SDO events. This indicate that Saharan dust advection is a natural source of particles having a lower specific OP with respect to the other sources acting on the area. Results suggest that OP could be an useful quantitative indicator to be used in epidemiological studies, in addition to PM mass concentrations, in regions frequently affected by high pollution events due to Saharan dust advection.

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Synthesis, characterization and electrochemical activity of α-MoO$_3$/Pt modified electrode to methanol oxidation

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The MoO$_3$ nanostructures have been synthesized by various methods, including pulsed laser deposition, thermal evaporation, RF magnetron sputtering sol–gel, hydrothermal, spray pyrolysis, chemical vapour deposition, mechanical exfoliation, molecular beam epitaxy and electrodeposition. These research works have revealed that MoO$_3$ exists as one of three polymorphic forms: a thermodynamically stable orthorhombic phase, a metastable monoclinic phase and a metastable hexagonal-phase. Many reports suggest that the majority of the MoO$_3$ materials are in the orthorhombic phase, the monoclinic phase or their mixtures (1,2).

In this communication we reported the synthesis of MoO$_3$ microplates by thermally oxidizing molybdenum foils at ambient atmosphere through resistive heating with no participation of templates or catalysts. Grown microplates MoO$_3$ structures were characterized by using scanning electron microscopy (SEM), transmission electron microscopy (TEM) coupled with selected area diffraction pattern (SAD), X-ray powder diffraction (XRD), micro-Raman and photoluminescence (PL) spectroscopy. The MoO$_3$ microplates synthesized after 60 min resistive heating, that have orthorhombic single-crystalline structures grown preferentially along the [001] direction. They are about 100-200nm in thickness and a few tens micrometers in length. As heating time proceeds to 80 min, plates of α-MoO$_3$ form a branched structure. A more attentive look shows that a primary plates formed at until 60 min could serve as substrates for the subsequent growth of secondary belts.

The electrocatalytic activity of α-MoO$_3$ plates on Pt electrodes was carried out by Cyclic Voltammetry (CV) in neutral phosphate buffer to investigate the electrochemical properties of α-MoO$_3$/Pt modified electrode against methanol oxidation. Electrochemical characterization of MoO$_3$ hierarchical structures highlighted the involvement of Mo(IV)/Mo(VI) species in electrodic processes at neutral pH, being diffusion controlled in oxidation and surface controlled in reduction. Moreover, the results obtained for electrochemical oxidation of methanol by Pt electrodes modified with MoO$_3$ structures showed the enhanced catalytic properties of Pt when acting in synergy with MoO$_3$ plate structures (3).

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Model of the electrical response of anti-thrombin TBA aptamer in bio-sensing applications

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Medicine is recently focusing on DNA-based treatments to improve prevention, personalized therapies and to achieve a less invasive and early diagnosis. Accordingly, researchers are devoted to design and produce a novel kind of macromolecules, the aptamers, single-stranded DNA and RNA sequences artificially produced to bind, with high affinity, the assigned ligands. Their mechanism of ligand binding is quite similar to the recognition process used by monoclonal antibodies, finding applications in therapies covering illnesses from cancer to cardiovascular diseases. Among aptamers, the DNA 15-mer TBA is one of the best known, both in structure and function. Its therapeutic action, related to the ability of binding the exosite II of thrombin, produce an effective inhibition of platelet thrombus formation. High affinity with thrombin has selected this aptamer as the ultimate component of thrombin sensors. In particular, Cai (1) proposed a TBA-based label-free sensor, able to detect thrombin with high selectivity and sensitivity by using the Electrochemical Impedance Spectroscopy technique with an electrode TBA functionalized. A sensitive change (up to about 200%) in the electrochemical impedance upon enzyme–aptamer complex formation was revealed. Some other data concerning structure and function of TBA came from X-ray spectroscopy (2). In particular, the structure of the aptamer-enzyme complex has been resolved in the presence of $K+$ and $Na+$. The different effects in thrombin inhibition due to the presence of these ions seem to be related to subtle differences in the aptamer-thrombin complex (2).

In this contribution we use the theoretical approach known as proteotronics (3) to set up a microscopic model of the electrical response of TBA alone and complexed with thrombin. The macromolecule is mapped into a network, by using an all-nucleobase/amino acid coarse grained procedure. Each node of the network corresponds to a single nucleobase/amino acid and contains three main data: the position of the C1 atom (C□), the resistivity and the polarizability. Two nodes are connected only if their physical distance is smaller than an assigned cut-off radius, RC. Each link mimics an elementary electrical impedance, that in this case, is an RC parallel circuit, in agreement with the experimental results. TBA has been analyzed in 3 different configurations: a. native state, b. complex state with $K+$ or $Na+$ c. active state. The calculated electrical resistance of the network describing the native structure of TBA is compared with those of the networks of the complex in the presence of both $K+$ and $Na+$. The complex shows a relevant improvement of resistance with respect the TBA alone, as observed in experiments, particularly in the presence of $K+$. This result nominates resistance measurement as a novel test of aptamer-ligand affinity. The capture of thrombin produces a change of TBA free energy which is described as a change of the interaction radius, RC, used for building the network. Finally, by analyzing the mechanism of TBA-thrombin conjugation and considering that dose-response observed in experiments depends on the fraction of TBAs which capture the thrombin, we are able to reproduce the experimental data and the agreement is quite good.

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Smartphone-based cell biosensors: a new tool for rapid on-site detection of chemical agents

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The routine monitoring of water, food, and the environment for chemical and biological threat agents is hampered by the fact that available techniques usually require clean samples and sophisticated equipment. Alternatively, unconventional analytical tools that allow rapid and low-cost monitoring of complex matrices are provided by biosensors. In particular, cell biosensors present the important feature of assessing the biological effects of a target analyte and its bioavailability. All chemical and biological threat agents share the ability to interact with molecular targets (e.g., receptors, membranes..) and therefore cells can be employed as "living sentinels". In the frame of the project "Cell biosensors for detection of chemical and biological threats", funded by NATO-SPS, we developed a battery of bioengineered cell biosensors "tailored" to respond to different agents by the generation of measurable and quantitative dose-dependent bioluminescent signals. As first proof-of-concept, *S. cerevisiae* yeast cells were bioengineered to respond to toxicants and endocrine disrupting chemicals acting via estrogen/androgen receptors. The bioengineered cells were integrated into 3D printed ready-to-use cartridges and low-cost smartphone adaptors were created using a desktop 3D printer to provide a mini-darkbox. Preliminary results will be presented about analytical performance of the smartphone-based device and its suitability for on-site surveillance.

Soil's fingerprinting for forensic applications: GC-MS as an useful tool

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The unique identification of soils is mandatory in several forensic investigations, as in the case of land under shoes of a murder victim to match the soil at the crime scene.

Different approaches have been proposed up to now to investigate this problem: among them, microscopic techniques, genome study of the bacterial flora in the soils, and chemical techniques that mostly rely on the determination of trace elements in the samples, usually by ICP or neutron activation analysis. In this study, different soils have been characterized for the volatile and semi-volatile organics. To this purpose, forest lands, farmlands, burnt ground, urban borders and grass, have been collected, characterized in terms of N content, COD, TOC, dry residue at 550°C, pH, etc, and analyzed by GC-MS. Different sample treatments have been considered to find organics that could be identified as fingerprint of a specific soil, from the headspace analysis to the solvent extraction prior and after pyrolysis of the soil (at 200°C), to the analysis of sugars, organic acids and similar compounds after derivatization of the soil extract with N,O-Bis(trimethylsilyl)trifluoroacetamide/trimethychlorosilane (1).The organics identified (polycyclic hydrocarbons, pesticides, sugars and terpenic compounds) are evaluated with the aim to assign some of them, whenever possible, to a certain typology of soil.

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Calabretta Maria Maddalena	ANA-PO88	Carlà Francesco	ANA-OR46	
	ANA-PO139	Carlucci Giuseppe	ANA-PO49	
Calabria Donato	ANA-PO85	Carlucci Maura	ANA-PO49	
Calabria Donato*	ANA-OR37	Caroli Antonio*	ANA-PO42	
Caliceti Cristiana*	ANA-PO85	Carretti Emiliano	ANA-PO103	
Caliceti Cristiana	ANA-OR37	Caruso Tonino*	ANA-PO11	
Callegher Bruno	ANA-PO01	Caruso Tonino*	ANA-OR01	
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Cecconi Daniela*	ANA-OR25		ANA-PO120
Ceglie Andrea	ANA-PO44	Cioffi Nicola*	ANA-KN09
Cellucci Luana	ANA-PO127	Cioffi Nicola*	ANA-PO89
Cenci Lucia*	ANA-PO87	Ciotoli Eleonora	ANA-PO109
Cenci Lucia	ANA/MAS-OR02	Ciriello Rosanna	ANA-PO07
Cennamo Nunzio	ANA-OR18	Clemente Maurizio	ANA-OR44
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Certomà Ilenia	ANA-PO108	Colombi Cristina	ANA-OR33
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Chelazzi David	ABC/ANA/FIS-OR01		ANA-PO80
Chenet Tatiana	ANA-PO12	Conca Eleonora	ANA/ELE/GS-OR03
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Chiaravalle A. Eugenio	ANA-PO52		ANA-PO108
Chiari Massimo	ANA-OR34		ANA-PO27
Chiariello Carmela	ANA-PO71	Conca Eleonora*	ANA-OR09
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Chieppa Marcello	ANA-PO55	Consolo Mariella	ANA-PO132
Chillè Donatella*	ANA-PO28	Contado Catia	ANA-PO45

Contado Catia*	ANA-OR24	Curci Alessandra	ANA-PO89
Conte Amalia	ANA-OR44	Curini Roberta	ANA-OR07
Conte Eleonora	ABC/ANA/FIS-OR07		ANA-PO127
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	ANA-PO71	D´Alessio Pasquale	ANA-PO131
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Contini Matteo	ANA-OR32	D'Ulivo Alessandro	ANA-PO112
Contursi Michela	ANA-OR39	Dalcanale Enrico	ANA-PO92
	ANA-PO90	Dale Nicholas	ANA/ELE/GS-OR02
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Cordoni Davide	ANA-PO16	Dall'Ara Alice	ANA-PO112
Corradini Claudio	ANA-PO76	Dalle Carbonare Luca	ANA-PO70
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Corradini Danilo*	ANA-PO46	Damiano Fabrizio	ANA-PO68
Corradini Roberto	ANA-PO92	D'Andrea Luca Domenico	ANA-OR17
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Coviello Donatella*	ANA-PO90		ANA-PO107
Coviello Donatella*	ANA-OR39	De Gara Laura	ANA-PO46
Cozzi Giulio	ANA-PO14	D'Elia Marcello	ANA-PO139
Crea Francesco	ANA-OR16	De Laurentiis Francesco	ANA-PO124
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Crescenzi Carlo	ANA-PO42	De Luca Domenico A.	ANA-PO20
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	ANA-OR26	De Luca Silvia	ANA-PO109
Crescenzi Carlo*	ANA-OR22	De Maria Letizia	ANA-OR43
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	ANA-PO68	
Filippo Emauela	ANA-PO137	
Filon Larese Francesca	ANA-OR06	
Fiore Luca	ANA-OR38	
Firmani Patrizia*	ANA-PO115	
Foglia Patrizia	ANA-PO43	
Fondi Marco	ANA-PO68	
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Foti Claudia	ANA-PO28	
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Franco Placido	ANA-PO73	
Franco Placido*	ANA-PO04	
Frascati Francesco	ANA-PO102	
Frasconi Marco	ANA-OR15	
	ANA-PO133	
Freschi Stefano	ANA-PO95	
Fuoco Roger	ANA-PO38	
	ANA-PO63	
Furia Emilia*	ANA-OR14	
Furia Emilia*	ANA-PO29	
Furlanetto Sandra	ANA-OR03	
	ANA-PO50	
Furlanetto Sandra*	ANA-PO51	
Furlanetto Sandra*	ANA-PO116	
Fusco Giovanni	ANA-PO91	
Fusco Sandra	ANA-PO35	
Gabriele Serena	ANA-OR41	
Gabrieli Jacopo	ANA-PO14	
Gaeta Carmine	ANA-PO11	
Gaiero Giulia	ANA-PO17	
Gallo Monica	ANA-PO59	
Gallo Vito*	ANA-PO135	
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Gans Peter	ANA-PO30	
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	ANA/MAS-OR06	
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	ANA-PO47	
Gattuso Giuseppe	ANA-OR16	
Genga Alessandra*	ANA-PO18	
Genga Alessandra*	ABC/ANA-KN03	
Gennaro Armando	ANA-OR19	
Genovese Cristina	ANA-PO09	
Gerace Enrico	ANA-PO64	
Germinario Giulia	ANA-PO06	
Ghimenti Silvia	ANA-PO38	
	ANA-PO63	
	ANA-PO100	
Giaccherini Andrea	ANA-OR46	
	ANA-PO95	
	ANA-PO102	
	ANA-PO120	
Giacobello Fausta*	ANA-PO30	
Giacomino Agnese	ANA/ELE/GS-OR03	
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	ANA-PO17	
	ANA-PO108	
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Giannarelli Stefania	ANA-PO39	
Giannetti Vanessa	ANA-PO134	
Giannetto Marco*	ANA-PO92	
Giannetto Marco*	ANA-PO93	
Giannetto Marco*	ANA-PO94	
Giannetto Marco*	ANA-KN05	
Giannini Francesca	ANA-PO117	
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	ANA/ELE/GS-OR05	Grotti Marco		ABC/ANA-OR05
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	ANA-PO138	Grotti Marco*		ANA-KN07
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Girault Hubert H.	ANA/ELE/GS-KN02			ANA-PO85
Girelli Domenico	ANA-PO86			ANA-PO106
Girelli Anna Maria	ANA-PO61	Guarnieri Sonia		ANA-PO140
Giubilato Elisa	ABC/ANA-KN01	Guascito Maria Rachele*		ANA-PO136
Giuffrè Ottavia	ANA-PO30	Guascito Maria Rachele*		ANA-PO137
Giuffrida Daniele	ANA-KN04	Guascito Maria Rachele*		ANA-PO138
Giuliano Mattia	ANA-PO108	Guella Graziano		ANA/MAS-OR02
Giurlani Walter*	ANA-PO95	Gufoni Stefano		ANA-PO100
Giurlani Walter	ANA-OR46	Guglielmetti Chiara		ANA-OR05
Glaciale Marco	ANA/MAS-KN01	Guglielmino Riccardo		ANA-PO03
	ANA-PO65	Gullifa Giuseppina*		ANA-PO69
Glavnikc Vesna	ANA-PO46	Gullifa Giuseppina		ANA-PO125
Gnagnarelli Valeria	ANA-PO16			ANA-PO126
Göbel Gero	ANA-PO91	Gurdeniz Göezde		ANA-OR02
Gómez-Amo Jose-Luis	ANA-KN03	Herniman Julie		ANA-PO04
Gorton Lo	ANA-OR20	Hibino Yuya		ANA-PO82
	ANA-OR40			ANA-PO83
	ANA-PO82	Hokke Cornelis H.		ANA/MAS-OR01
	ANA-PO83	Holmbäck Jan		ANA-PO56
Gorroni Agnese	ANA-PO140			ANA-OR26
Gosetti Fabio	ABC/ANA/FIS-OR07	Holmgren Erik		ANA-OR22
	ANA-PO10	Hooshyaria Maryam		ANA-PO116
	ANA-PO23	Hugelier Siewert		ANA-PO115
	ANA-PO24	Hushpulian Dmitry		ANA-OR20
	ANA-PO70	Iadaresta Francesco		ANA-OR22
	ANA-PO71	Iadaresta Francesco*		ANA-OR26
	ANA-PO121	Iadaresta Francesco*		ANA-PO56
	ANA-PO122	Idili Andrea		ANA-KN08
Gosetti Fabio*	ANA-PO19			ANA-PO101
Gosetti Fabio*	ANA-KN06	Illiano Anna*		ANA-PO132
Gosetti Fabio*	ANA-PO20	Illuminati Silvia		ANA-PO27
Granafei Sara	ANA-PO75	Inferrera Veronica		ANA-KN04
Grasso Giuliana	ANA-OR22	Innocenti Massimo		ANA-PO50
	ANA-PO42			ANA-PO95
Gregori Adolfo	ANA-OR07			ANA-PO102

	ANA-PO120	Leech Donal	ANA-OR20
Innocenti Massimo*	ANA-OR46	Legrand Stjin	ABC/ANA/FIS-KN01
Intini Nicola	ANA-PO135	Lesch Andreas*	ANA/ELE/GS-KN02
Irto Anna	ANA-OR16	Lesch Andreas	ANA-PO113
Irto Anna*	ANA-PO32	Li Donghao	ANA-PO57
Isca Clelia	ABC/ANA/FIS-OR06		ANA-PO58
Isernia Carla	ANA-OR17	Li Qianjin	ANA-PO42
Ismail Omar H.	ANA-OR12	Licandro Emanuela	ANA-OR19
	ANA-PO47		ANA-PO81
Isse Abdirisak Ahmed	ANA-OR19		ANA-PO99
Iuliano Mauro	ANA-OR17	Lisdat Fred	ANA-PO91
Iurlo Matteo	ANA/ELE/GS-OR05	Lista Florigio	ANA/ELE/GS-OR07
Izzi Margherita	ANA-OR44	Lo Re Alessandra	ABC/ANA-OR02
Izzo Simona	ANA-PO110	Lo Sterzo Claudio	ANA-PO60
Janssens Koen	ABC/ANA/FIS-KN01	Lomonaco Tommaso	ANA-PO38
Kano Kenji	ANA-PO82		ANA-PO63
	ANA-PO83		ANA-PO100
Koehler Brigit	ABC/ANA-KN02	Lopez Francesco	ANA-PO44
Koivula Anu	ANA-OR40	López-Lorente Ángela I.	ANA-KN09
Kornejew Eckhart	ANA-PO91	Lopreside Antonia	ANA-PO88
Kranz Christine	ANA-KN09	Lopreside Antonia*	ANA-PO139
La Barbera Giorgia	ANA-PO43	Losito Ilario*	ANA-OR21
	ANA-PO48	Losito Ilario	ANA-PO75
La Barbera Giorgia*	ANA-OR02	Lucarelli Franco	ANA-KN03
La Bianca Mariacristina	ANA-OR19		ANA-OR34
La Nasa Jacopo	ABC/ANA/FIS-KN02	Łucejko Jeannette Jacqueline	ABC/ANA/FIS-OR10
Lachowicz Joanna Izabela*	ANA-PO33	Lucentini Luca	ANA-KN01
Laganà Aldo	ANA-OR11	Ludwig Roland	ANA-OR40
	ANA-OR42	Lupi Angelo	ANA-OR34
	ANA-PO43		ABC/ANA-OR05
	ANA-PO48	Ma Biao	ANA-PO58
	ANA-PO66	Macii Francesca	ANA-PO39
Landelius Tomas	ABC/ANA-KN02	Maggi Maria Anna	ANA-PO15
Lando Gabriele	ANA-OR16		ANA-PO114
	ANA-PO84	Magi Emanuele	ANA-OR29
Lando Gabriele*	ANA-PO34	Magi Emanuele*	ANA-PO77
Langerame Fausto	ANA-PO07	Magnacca Giuliana	ANA-PO20
Langley John	ANA-PO04	Magnani Luca	ANA-OR05
Lannuzel Delphine	ANA-PO09	Magrì Andrea	ANA-OR47
Lasagna Manuela	ANA-PO20		ANA-PO109
Latronico Mario	ANA-PO135	Maietti Annalisa	ANA-OR04
Latruwe Kris	ANA-KN07	Maitane Olivares	ABC/ANA/FIS-KN02
Lavacchi Alessandro	ANA-PO95	Malacaria Luana	ANA-OR14

Malacrida Claudia	ANA-PO99		ANA-PO37
Malandrino Mery	ANA-PO27	Marassi Valentina*	ANA-OR13
	ANA/ELE/GS-OR03	Marcaccio Massimo	ANA/ELE/GS-OR05
	ANA-OR09		ANA-OR20
	ABC/ANA-OR05	Marchesani Giuliana*	ANA-PO52
	ANA-PO17	Marchetti Alessia	ANA-PO79
	ANA-PO108	Marchetti Nicola*	ANA-OR04
	ABC/ANA-OR01	Marchetti Simone	ANA-OR18
Malaspina Fabrizio	ANA-PO129	Marchetti Simone*	ANA-OR43
Malavasi Lorenzo	ANA-OR32	Marchiandi Sergio	ANA-PO105
	ANA-PO36	Marcomini Antonio*	ABC/ANA-KN01
Malegori Cristina	ANA-PO05	Marengo Emilio	ANA-OR05
	ANA-PO118		ABC/ANA/FIS-OR07
	ANA-PO119		ANA-OR25
Malegori Cristina*	ANA-OR48		ANA-OR49
Malgieri Gaetano	ANA-OR17		ANA-PO10
Malitesta Cosimino	ABC/ANA-KN03		ANA-PO19
	ANA-PO18		ANA-PO20
	ANA-PO42		ANA-PO23
	ANA-PO97		ANA-PO70
Mancini Ignazio M.	ANA-OR30		ANA-PO71
Manera Maria Grazia	ANA-OR23		ANA-PO121
Manfra Michele	ANA-PO55		ANA-PO122
	ANA-PO62	Margiotta Nicola	ANA-PO89
Manfredi Carla *	ANA-PO35	Mariani Federica	ANA/ELE/GS-OR01
Manfredi Marcello*	ANA-PO70	Mariani Stefano	ANA-PO97
Manfredi Marcello*	ANA-PO71	Marinelli Tecla	ANA-OR30
Manfredi Marcello	ABC/ANA/FIS-OR07	Marini Federico	ANA-OR47
	ANA-OR25		ANA-PO29
	ANA-OR49		ANA-PO109
	ANA-PO10		ANA-PO110
	ANA-PO19		ANA-PO111
	ANA-PO20		ANA-PO115
	ANA-PO23	Marletti Matteo	ABC/ANA-OR01
	ANA-PO24	Marrazza Giovanna*	ANA-PO96
	ANA-PO121	Marrazza Giovanna*	ANA-PO22
	ANA-PO122	Martellini Tania	ANA-PO22
Manfredi Marcello*	ANA-OR05	Marti Matteo	ANA-OR07
Mangiacotti Michele	ANA-PO52	Martinelli Nicola	ANA-PO71
Manicardi Alex	ANA-PO92	Martyna Agnieszka	ANA-PO128
Manniello Michele Dario	ANA-OR26	Marzani Luca	ANA-PO22
Mantini Caterina	ANA-PO27	Marzilli Mario	ANA-PO38
Maraschi Federica	ANA-OR32	Marzio Rosi	ANA/MAS-OR06

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Mascherpa Marco Carlo	ANA-PO112		ANA-PO57
Mascini Marcello	ANA-PO80		ANA-PO58
Masi Salvatore	ANA-OR30		ANA-PO78
Massi Alessandro	ANA-PO85	Mercogliano Paola	ABC/ANA-KN01
Massoud Renato	ANA/ELE/GS-KN01	Merli Daniele*	ANA-PO140
	ANA-OR38	Merli Daniele	ANA-OR43
Mastrangelo Rosangela	ABC/ANA/FIS-OR03	Merlo Francesca	ANA-OR32
Mastrocinque Raffaella	ANA-PO62	Mezgec Karin	ABC/ANA-OR04
Mastrorilli Piero	ANA-PO135	Micheli Laura*	ANA-PO117
Mastrorocco Antonella	ANA/MAS-KN01	Micheli Laura	ANA-PO21
Materazzi Stefano	ANA-PO125	Michelini Elisa	ANA-PO88
	ANA-PO126		ANA-PO139
	ANA-PO69	Mihali Voichita	ANA-OR19
Mattarozzi Monica	ANA-PO67	Milanese Chiara	ANA-PO36
	ANA-PO92	Milano Francesco	ANA-PO138
	ANA-PO93	Milea Demetrio	ANA-OR16
	ANA-PO94	Milica Jovic	ANA/ELE/GS-KN02
Mattonai Marco	ANA-PO79	Minella Marco	ABC/ANA-OR02
MattorreBenedetta	ANA-PO68		ABC/ANA-KN02
Mauro Marcella	ANA-OR06	Minella Marco*	ANA-OR45
Mayer Alice	ANA-PO24	Minero Claudio	ABC/ANA-KN02
Mazza Maria	ANA-OR05		ANA-OR45
Mazzaracchio Vincenzo*	ANA/ELE/GS-OR07	Miniotti Eleonora	ANA-PO24
Mazzei Franco	ANA-OR20	Minunni Maria	ANA-OR23
	ANA-OR40		ANA-PO103
	ANA-PO91		ANA-PO104
Mazzeo Rocco*	ABC/ANA/FIS-KN01	Mirabile Antonio	ABC/ANA/FIS-OR02
Mazzeo Rocco	ANA-PO05		ANA-PO06
Mazzola Mauro	ANA-OR34	Mirasole Cristiana	ANA-OR29
	ABC/ANA-OR05	Mirasoli Mara*	ANA-PO98
Mazzone Giuseppina	ANA-OR30	Mirasoli Mara	ANA-KN02
Mazzotta Elisabetta*	ANA-PO97		ANA-OR37
Mazzotta Lorenzo	ANA-PO03		ANA-PO113
Mazzuca Claudia	ANA-PO117		ANA-PO85
Mazzucco Eleonora	ANA-PO71		ANA-PO106
	ANA-PO122	Mirasoli Mara	ANA-PO107
Mazzucco Eleonora*	ANA-PO23	Mistri Michele	ANA-PO12
Mazzucco Eleonora*	ANA-PO24	Mizaikoff Boris	ANA-KN09
Meier Florian	ANA-PO45	Močnik Grisa	ABC/ANA-OR07
Melfi Maria Teresa*	ANA-PO53	Modugno Francesca	ABC/ANA/FIS-OR10
Meloni Daniela	ANA-KN03	Mondello Luigi	ANA/MAS-KN02
Melucci Dora	ANA-PO124		ANA-KN04
Mentana Annalisa	ANA-PO53	Montalbano Letizia	ABC/ANA/FIS-OR02

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Montegrossi Giordano	ANA-PO95		Neagu Daniela	ANA/ELE/GS-OR06
Montesano Camilla	ANA-OR07			ANA/ELE/GS-OR07
	ANA-PO16		Neri Placido	ANA-PO11
	ANA-PO127		Netti Paolo Antonio	ANA-PO59
Monticelli Damiano*	ANA-PO130		Nicolardi Simone*	ANA/MAS-OR01
Montis Costanza	ABC/ANA/FIS-OR03		Nicoletti Isabella	ANA-PO46
Montone Carmela Maria	ANA-OR42		Nicolis Stefania	ANA-PO37
	ANA-PO48		Nisi Stefano	ANA-PO114
	ANA-PO66		Notardonato Ivan*	ANA-OR28
Montone Carmela Maria*	ANA-OR11		Notardonato Ivan*	ANA-PO25
Montoya Noemì	ABC/ANA/FIS-OR05		Novellino Ettore	ANA-PO62
Mora Paolo	ANA-PO67		Nurchi Valeria Marina	ANA-PO33
Moretti Sacha	ANA-OR27		Olivares Maitane	ABC/ANA/FIS-KN02
Moro Isabella	ANA-PO40		Oliveri Paolo	ANA-OR48
Moroni Beatrice	ABC/ANA-OR05			ABC/ANA/FIS-KN01
Moscone Daniela	ANA/ELE/GS-OR07			ANA-PO118
	ANA/ELE/GS-KN01			ANA-PO119
	ANA/ELE/GS-OR06		Oliveri Paolo*	ANA-PO05
	ANA-OR08		Olivieri Oliviero	ANA-PO71
	ANA-OR38		Onida Barbara	ABC/ANA-OR02
Motta Oriana	ABC/ANA-OR07		Onor Massimo	ANA-PO41
Mussini Patrizia Romana*	ANA-PO99			ANA-PO112
Mussini Patrizia Romana	ANA-PO81		Onor Massimo*	ANA-PO100
Mussini Patrizia Romana*	ANA-OR19		Orbelli Biroli Alessio	ANA-PO99
Naccarato Attilio	ANA-PO74		Orlandi Marco*	ABC/ANA/FIS-OR10
Naccarato Attilio*	ANA-OR27		Orlandini Serena	ANA-OR03
Napoli Anna	ANA-PO30			ANA-PO50
Nardiello Donatella	ANA-PO53			ANA-PO51
	ANA-PO57			ANA-PO116
	ANA-PO58		Orlando Ilaria	ANA-PO89
	ANA-PO78		Ornaghi Paola	ANA-PO86
Nascetti Augusto	ANA-PO98		Orsini Francesca	ANA-PO46
	ANA-PO107		Ortiz Maria Cruz	ANA-PO116
Natale Anna	ANA-PO53		Ostacolo Carmine	ANA-PO55
	ANA-PO78			ANA-PO62
Natale Gabriella	ANA-OR19		Östmana Conny	ANA-OR26
	ANA-PO99		Pace Giandomenico	ANA-KN03
Natile Giovanni	ANA-PO89		Pacifici Laura	ANA-PO129
Nava Silvia	ANA-KN03		Paciulli Maria	ANA-PO86
Navarra Gabriele	ABC/ANA/FIS-OR09		Padoan Sara*	ABC/ANA-OR03
Naviglio Daniele*	ANA-PO54		Pagano Francesco*	ANA-PO55
Naviglio Daniele*	ANA-PO72		Pagano Francesco	ANA-PO62
Naviglio Daniele	ANA-PO59		Palazzo Gerardo	ANA-KN09

Palchetti Ilaria*	ANA-OR36	Peano Clelia	ANA-PO68
Palermo Carmen	ANA-PO53	Pedrini Alessandro	ANA-PO92
	ANA-PO78	Pelacani Samanta	ANA-PO123
	ANA-PO58	Pelicci Pier Giuseppe	ANA/ELE/GS-OR02
	ANA-PO57		ANA/ELE/GS-OR05
Palladino Pasquale*	ANA-OR23	Pellegrini Marika	ANA-PO60
Palleschi Giuseppe	ANA/ELE/GS-KN01	Peluso Andrea	ANA-PO35
	ANA-KN08	Penicaud Alain	ANA-PO113
	ANA/ELE/GS-OR04	Pennetta Antonio	ABC/ANA/FIS-OR08
	ANA/ELE/GS-OR07		ANA-PO03
	ANA-OR08	Pepe Giacomo	ANA-PO55
	ANA-OR10		ANA-PO62
	ANA-OR35	Pepi Federico	ANA/MAS-OR04
	ANA-OR38		ANA/MAS-OR06
	ANA-PO21	Pesavento Maria	ANA-OR43
	ANA-PO117	Pesavento Maria*	ANA-OR18
Palma Pierangela	ANA/MAS-OR05	Pesce Marco	ABC/ANA-KN01
Palmisano Francesco	ANA/MAS-KN01	Petkov Boyan	ANA-OR34
	ANA-OR21	Petranich Elisa	ANA-PO08
	ANA-PO65	Petropoulos Konstantinos*	ANA-PO21
	ANA-PO75	Picca Rosaria Anna	ANA-OR46
Paolucci Francesco	ANA/ELE/GS-OR02		ANA-OR44
	ANA/ELE/GS-OR05	Picca Rosaria Anna	ANA-KN09
Parisi Emmanuele *	ANA-PO35	Picca Rosaria Anna*	ANA-PO120
Pascale Emanuela	ANA-PO104	Piergiovanni Maurizio	ANA/MAS-OR05
Pascale Raffaella*	ANA-OR30	Piermarini Silvia	ANA/ELE/GS-OR04
Pasquini Benedetta	ANA-PO50	Piermarini Silvia	ANA-PO21
	ANA-PO51	Pifferi Valentina	ANA-OR36
	ANA-PO116	Pinalli Roberta	ANA-PO92
Passacantando Maurizio	ANA-PO15	Pinatel Eva	ANA-PO68
Passaponti Maurizio	ANA-PO102	Pinto Gabriella*	ANA-PO131
Pasti Luisa	ANA-OR04	Piovesana Susy	ANA-OR42
	ANA-PO12		ANA-PO43
Pastore Paolo	ANA-KN01		ANA-PO66
	ANA-OR15	Piovesana Susy*	ANA-IN01
	ANA-PO40	Pironti Concetta	ABC/ANA-07
	ANA-PO133	Pirro Valentina	ANA-PO128
Pastorini Graziella	ANA-OR48	Pirrone Nicola	ANA-OR27
Pastran Domingo	ANA-OR43	Pittia Paola	ANA-PO60
Paolucci Francesco	ANA-PO113		ANA-PO80
Pavoni Elena	ANA-PO08	Pitzalis Emanuela	ANA-PO112
Pazzi Marco	ANA-PO17	Pizzol Lisa	ABC/ANA-KN01
	ANA-PO129	Plaxco Kevin W.	ANA-KN08
Peana Massimiliano	ANA-PO33		

Poggi Giovanna	ABC/ANA/FIS-OR04	Ribechini Erika*	ANA-PO79
Poli Stefano	ANA-OR31	Riboni Nicolò	ANA-PO67
Pomponi Alice	ANA/ELE/GS-OR07	Ricci Andreina*	ANA/MAS-OR04
Porchetta Alessandro	ANA/ELE/GS-OR07	Ricci Andreina	ANA/MAS-OR06
	ANA-OR10	Ricci Francesco	ANA-KN08
Porchetta Alessandro*	ANA-OR35		ANA-IN03
Porru Emanuele*	ANA-PO73		ANA-PO101
Porru Emanuele	ANA-PO85		ANA-OR35
Prati Silvia	ABC/ANA/FIS-KN01	Ricci Francesco*	ANA-PL02
	ANA-PO05	Rienzner Michele	ANA-PO24
Praticò Giulia	ANA-OR02	Righetti Pier Giorgio	ABC/ANA/FIS-OR07
Prenesti Enrico	ANA-PO01	Rinaldi Massimiliano	ANA-PO86
Presta Marco	ANA-PO29	Risoluti Roberta	ANA-PO69
Prévost-Tremblay Carl	ANA-KN08		ANA-PO125
	ANA-PO101	Risoluti Roberta*	ANA-PO126
Profumo Antonella	ANA-OR32	Rivaro Paola	ANA-PO09
	ANA-PO36	Rivoira Luca*	ABC/ANA-OR02
	ANA-PO140	Rizzi Cristiana	ABC/ANA-OR07
	ANA-PO37	Rizzo Simona	ANA-OR19
Proto Antonio	ABC/ANA-KN03	Rizzuti Antonino	ANA-PO135
	ABC/ANA-OR07	Robotti Elisa	ABC/ANA/FIS-OR07
	ANA-PO18		ANA-PO10
Pucci Eleonora	ANA/ELE/GS-OR04		ANA-PO19
Quasso Fabio	ANA-PO23		ANA-PO20
	ANA-PO24		ANA-PO23
	ANA-PO122		ANA-PO24
Quinto Maurizio	ANA-PO53		ANA-PO70
Quinto Maurizio*	ANA-PO57		ANA-PO71
Quinto Maurizio*	ANA-PO58	Robotti Elisa*	ANA-PO121
Quinto Maurizio*	ANA-PO78	Robotti Elisa*	ANA-PO122
Ragone Rosa	ANA-PO135	Robotti Elisa*	ANA-OR49
Ramon Morales-lara Francisco	ANA-PO133	Rocchi Rachele	ANA-PO80
Ranallo Simona*	ANA-PO101	Roda Aldo	ANA-KN02
Ranallo Simona*	ANA-KN08		ANA-OR37
Ranzato Elia	ANA-PO121		ANA-PO04
Rapino Stefania	ANA/ELE/GS-OR02		ANA-PO73
	ANA-PO113		ANA-PO88
Rapino Stefania*	ANA/ELE/GS-OR05		ANA-PO139
Recchia Sandro *	ANA-OR31		ANA-PO98
Rella Roberto	ANA-OR23		ANA-PO106
Renai Lapo	ANA-OR03		ANA-PO113
Reschiglian Pierluigi	ANA-OR13		ANA-PO107
Ribechini Erika*	ABC/ANA/FIS-KN02		ANA-PO85

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Roda Barbara	ANA-OR13			ANA-PO32
Romanelli Alessandra	ANA-OR17			ANA-PO34
Romani Marco	ANA-PO23			ANA-PO84
	ANA-PO24		Săndulescu Robert	ANA-PO96
Rombolà Alessandro Girolamo*	ANA-PO26		Sannicolò Francesco	ANA-OR19
				ANA-PO81
Romolo Francesco Saverio	ANA-PO126			ANA-PO99
Rosato Roberta	ANA/MAS-OR03		Santini Monia	ABC/ANA-KN01
Rossetti Marianna	ANA-KN08		Sanvito Francesca	ANA-PO130
Rossetti Marianna	ANA-OR35		Sarabia Luis Antonio	ANA-PO116
Rossetti Marianna*	ANA-OR10		Sardanelli Anna Maria	ANA/MAS-KN01
Rossi Antonella	ABC/ANA/FIS-OR09		Scagliola Alessandra	ANA-PO121
	ANA-PO02		Scalabrini Andrea	ANA-PO36
Rossini Daniele	ANA-OR03		Scali Maria Chiara	ANA-PO38
Rostagno Chiara	ABC/ANA-OR07		Scapicchio Pasquale	ANA-PO135
Rozzi Andrea	ANA-PO92		Scarano Simona*	ANA-PO103
Ruckebusch Cyril	ANA-PO115		Scarano Simona*	ANA-PO104
Ruggieri Fabrizio	ANA-PO15		Scarano Simona	ANA-OR23
	ANA-PO114		Scarchilli Claudio	ABC/ANA-OR04
Ruo Redda Andrea	ANA/ELE/GS-OR03		Scarpone Rossana	ANA-PO16
	ANA-PO108		Scarpone Rossana*	ANA/MAS-OR03
Russo Francesca	ANA-PO102		Scarponi Giuseppe*	ANA-PO27
Russo Mario Vincenzo	ANA-OR28		Scavetta Erika*	ANA/ELE/GS-OR01
	ANA-PO25		Schepis Antonino	ANA/MAS-KN02
Russo Paola	ANA-OR26		Schiavone Sergio	ANA-PO125
Sabbatini Luigia*	ANA-PO06		Sciarrone Danilo*	ANA/MAS-KN02
Sacchetti Gianni	ANA-PO85		Sciuto Katia	ANA-PO40
Sadun Claudia	ANA-OR41		Sciutto Giorgia	ABC/ANA/FIS-KN01
Saiano Filippo*	ANA-PO123			ANA-PO05
Salanti Anika	ABC/ANA/FIS-OR10		Scordo Giorgio	ANA/ELE/GS-KN01
Salernitano Elena	ANA-PO112		Scrano Laura	ANA-PO07
Salomone Alberto	ANA-PO128		Scuto Francesca Romana	ANA-PO61
Salvadeo Paola	ANA-PO76		Secco Andrea	ANA-PO122
Salvatore Francesco	ANA-PO59		Sellergren Börje	ANA-PO42
Salvatore Maria Michela*	ANA-PO59		Selvolini Giulia	ANA-PO96
Salvi Anna Maria*	ANA-PO07		Sergi Manuel*	ANA-PO16
Salvietti Emanuele*	ANA-PO102		Sergi Manuel*	ANA-PO60
Salvietti Emanuele	ANA-OR46		Sergi Manuel*	ANA-PO80
	ANA-PO120		Sergi Manuel	ANA-OR07
Salvitti Chiara	ANA/MAS-OR04			ANA/MAS-OR03
Salvitti Chiara*	ANA/MAS-OR06			ANA-PO127
Salvo Pietro	ANA-PO100		Severi Mirko	ANA-KN03
Sammartano Silvio	ANA-OR16			ANA-OR34
	ANA-PO30			

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	ABC/ANA-OR05			ANA-OR27
Severi Mirko*	ABC/ANA-OR04		Tagarelli Antonio*	ANA-PO74
Sferlazzo Damiano Massimiliano	ANA-KN03		Takahama Satoshi	ANA-OR33
Shinde Sudhirkumar	ANA-PO42		Talà Adelfia	ANA-PO68
Siciliano Maria	ABC/ANA-KN03		Talotta Carmen	ANA-PO11
	ANA-PO18		Tamburrini Diego	ABC/ANA/FIS-OR10
Siciliano Tiziana	ABC/ANA-KN03		Tassone Antonella	ANA-OR27
	ANA-PO18		Tempesti Paolo	ABC/ANA/FIS-OR03
Siculella Luisa	ANA-PO68		Tepore Antonio	ANA-PO137
Silvestri Simone	ANA-PO23		Tepore Marco	ANA-PO137
	ANA-PO24		Termopoli Veronica*	ANA/MAS-OR05
Simeoni Maria Chiara	ANA-PO60		Tessarolo Marta	ANA/ELE/GS-OR01
Simoni Patrizia	ANA-OR37		Testini Mariangela	ANA-PO68
Sogne Vanessa	ANA-PO45		Testolin Anna	ANA-OR36
Soldà Alice	ANA/ELE/GS-OR05		Thomas Elizabeth R.	ABC/ANA-OR04
Soldà Alice*	ANA/ELE/GS-OR02		Thorsten Klein	ANA-PO45
Sommella Eduardo	ANA-PO55		Tinti Alessandro*	ANA-OR33
	ANA-PO62		Tiraboschi Carla	ANA-OR31
Soriente Annunziata	ANA-PO11		Todisco Stefano	ANA-PO135
Sorrentino Fiodor	ANA-PO100		Togna Anna Rita	ANA-OR07
Spadaccino Giuseppina	ANA-PO53		Tomassetti Mauro*	ANA-PO105
	ANA-PO57		Tomassetti Mauro	ANA-PO110
	ANA-PO58		Tomei Maria Rita*	ANA/ELE/GS-OR06
	ANA-PO78		Tommasini Simone	ANA-PO123
Speltini Andrea*	ANA-OR32		Tonelli Domenica	ANA/ELE/GS-OR01
Speltini Andrea*	ANA-PO36		Torelli Paola*	ANA-PO134
Speltini Andrea*	ANA-PO37		Torresan Silvia	ABC/ANA-KN01
Sportelli Maria Chiara	ANA-KN09		Torri Cristian	ANA-PO26
Sportelli Maria Chiara*	ANA-OR44		Torsi Luisa*	ANA-PL01
Sprovieri Francesca	ANA-OR27		Tortolini Cristina	ANA-OR40
Stenni Barbara	ABC/ANA-OR04		Tositti Laura	ANA-PO124
Stevanin Claudia	ANA-PO12		Trabace Maddalena	ABC/ANA/FIS-OR02
Stevar Daniela A.	ANA-OR40		Tranvik Lars J.	ABC/ANA-KN02
Stjin Legrand	ABC/ANA/FIS-KN01		Traversi Rita	ANA-KN03
Strafella Alessandra	ANA-PO112			ABC/ANA-OR04
Strambini Lucanos M.	ANA-PO97			ABC/ANA-OR05
Sturini Michela	ANA-OR32		Traversi Rita*	ANA-OR34
	ANA-PO37		Tricase Angelo	ANA-KN09
Tacchini Massimo	ANA-PO85		Trifuoggi Marco	ANA-PO131
Tafur Marinos Janeth	ANA-PO13			ANA-PO132
	ANA-PO31		Trinei Mirella	ANA/ELE/GS-OR05
Tafur Marinos Janeth*	ABC/ANA-OR06		Trivella Maria Giovanna	ANA-PO38
Tagarelli Antonio	ANA-OR14		Troiani Anna	ANA/MAS-OR04

	ANA/MAS-OR06	Vione Davide*	ABC/ANA-OR01	
Truzzi Cristina	ANA-PO27		ABC/ANA-KN02	
Tumiati Simone	ANA-OR31	Vitale Vito	ANA-OR34	
Ubaldi Paolo Giuseppe	ANA-OR05		ABC/ANA-OR05	
Udisti Roberto	ANA-KN03	Volino Sabato	ANA-PO35	
	ABC/ANA-OR04	Volpe Annalisa	ANA-OR44	
	ANA-OR09	Volpe Giulia	ANA/ELE/GS-OR04	
	ANA-OR34		ANA-PO21	
Udisti Roberto*	ABC/ANA-OR05	Volpi Giorgio	ANA-PO31	
Ungaro Fabrizio	ANA-PO123	Volpin Stefano	ABC/ANA/FIS-OR06	
Vaglio Sara	ANA-PO64	Vovk Irena	ANA-PO46	
Valenti Giovanni*	ANA-PO113	Wei Ouyang	ABC/ANA-KN01	
Valenti Maria Teresa	ANA-PO70	Wuhrer Manfred	ANA/MAS-OR01	
Valentini Antonio	ANA-KN09	Yiming Jia	ANA-PO05	
Vallée-Bélisle Alexis	ANA-KN08	Zadora Grzegorz	ANA-PO128	
		Zangheri Martina	ANA-OR37	
	ANA-PO101		ANA-PO113	
Valmacco Federico	ABC/ANA-OR01		ANA-PO98	
Van der Burgt Yuri E.M.	ANA/MAS-OR01	Zangheri Martina*	ANA-PO106	
Van der Werf Inez	ANA-PO06	Zangheri Martina*	ANA-PO107	
Vanhaecke Frank	ANA-KN07	Zangheri Martina*	ANA-KN02	
Vannutelli Gabriele*	ANA-PO127	Zangrando Roberta	ABC/ANA-OR03	
Vannutelli Gabriele	ANA-OR07	Zanoni Robertino	ANA-PO91	
Vardè Massimiliano	ANA-PO14	Zappi Alessandro*	ANA-PO124	
Vassura Ivano	ANA-PO26	Zappi Daniele*	ANA-OR41	
Vecchione Raffaele	ANA-PO59	Zattoni Andrea	ANA-OR13	
Venditti Francesco	ANA-PO44	Zelano Vincenzo	ABC/ANA-OR06	
Ventura Giovanni	ANA-PO65		ANA-PO13	
Ventura Giovanni*	ANA-PO75		ANA-PO31	
Venturini Elisa	ANA-PO26	Zenezini Chiozzi Riccardo	ANA-OR11	
Venzo Alfonso	ANA-OR15	Zenezini Chiozzi Riccardo*	ANA-OR42	
Vincenti Flaminia	ANA-PO127	Zeni Luigi	ANA-OR18	
Vincenti Flaminia*	ANA-OR07	Zerbetto Francesco	ANA/ELE/GS-OR05	
Vincenti Marco*	ANA-PO128	Zirino Alberto	ABC/ANA-KN01	
Vincenti Marco*	ANA-PO129	Zoia Luca	ABC/ANA/FIS-OR10	
Vincenti Marco	ANA-PO64	Zoroddu Maria Antonietta	ANA-PO33	
Viola Alessia	ANA-PO56	Zou Yilin	ANA-PO57	
Vione Davide	ABC/ANA-OR02			