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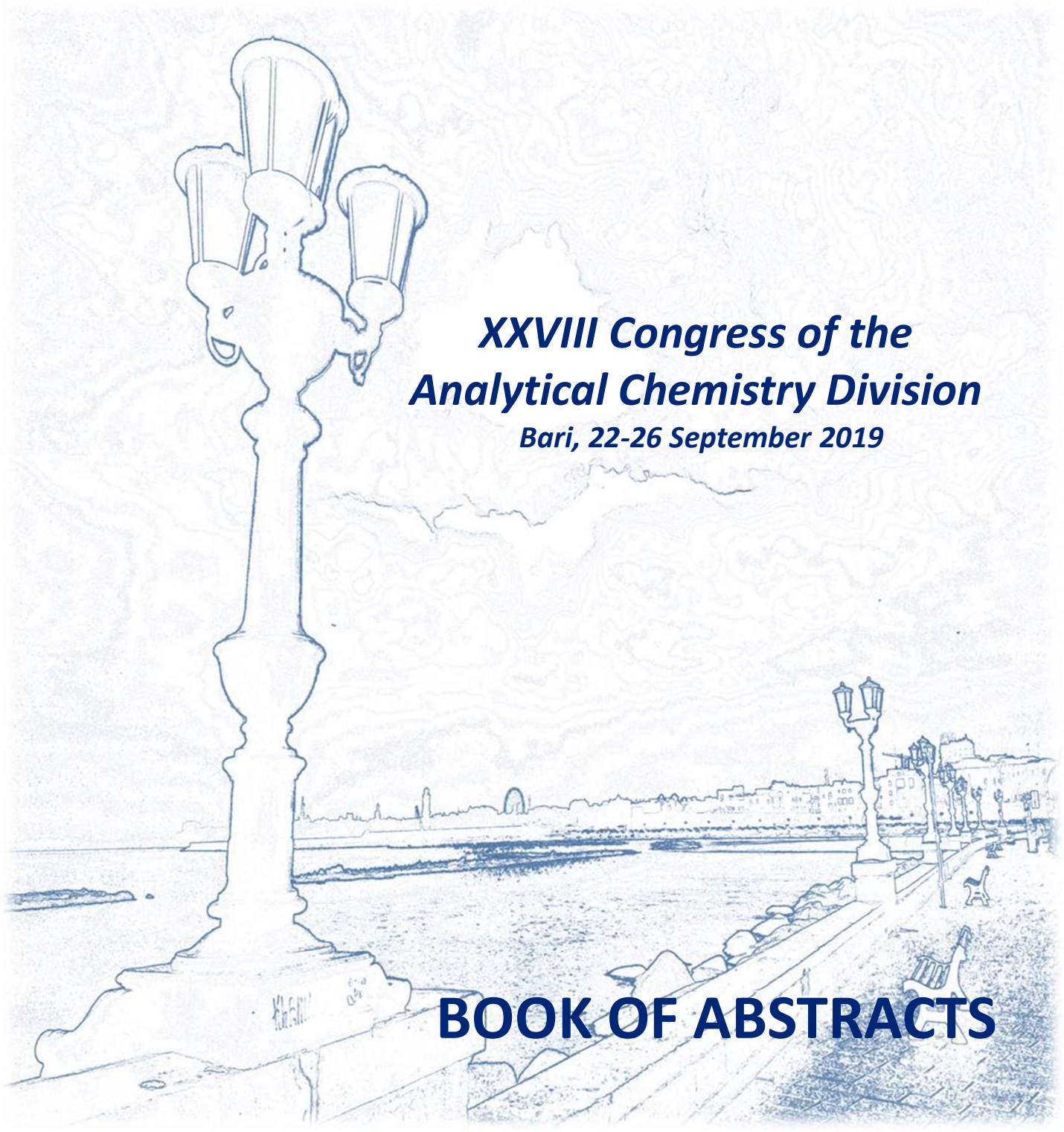

XXVIII Congress of the Analytical Chemistry Division

Bari, 22-26 September 2019

BOOK OF ABSTRACTS

Società Chimica Italiana

Book of Abstracts
XXVIII Congress of the
Analytical Chemistry Division

Bari 22 – 26 September 2019
Università degli Studi di Bari Aldo Moro

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PREFACE

The XXVIII Congress of the Analytical Chemistry Division, held in Bari at Università degli Studi di Bari Aldo Moro from 22 to 26 September 2019, has been attended by about 280 delegates from both the academic and research institutions. Particularly numerous was the participation of young researchers, also thanks to the far-sighted policy of our Division that funded 40 scholarships assigned on the basis of a national call.
To celebrate the 150th anniversary of the Periodic Table of the Chemical Elements, the Opening Ceremony ended with the *Lectio Magistralis* "Homo sapiens history and periodic table of elements: an inseparable pair", held by Professor Adriano Zecchina (*Accademico dei Lincei*) introduced by Professor Angela Agostiano, President of the Italian Chemical Society.
The scientific programme comprised 4 plenary lectures, 11 keynote lectures, 120 oral presentations and 131 poster presentations.
The following researchers have been awarded with Prizes and Medals of the Analytical Chemistry Division:

"Premio alla Carriera"– Prof. Aldo RODA, Alma Mater Studiorum Università di Bologna
"Medaglia Liberti"– Prof. Carlo BICCHI, Università degli Studi di Torino
"Medaglia Canneri"– Prof. Roberto TODESCHINI, Università degli Studi di Milano Bicocca
"Premio Giovane Ricercatore" – Dr Stefano CINTI, Università degli Studi di Napoli Federico II
"Premio di Laurea" – Miss Francesca RUSSO, Università degli Studi di Firenze

Moreover, 10 Best Poster Presentations prizes, sponsored by *ACS Omega* (ACS), *Analytical and Bioanalytical Chemistry* (Springer) e *Antibiotics* (MDPI), have been awarded to: Eleonora Amante (Università degli Studi di Torino), Sabrina Di Masi (Università del Salento), Ottavia Giampaoli (Sapienza Università di Roma), Antonio Gigliuto (Università degli Studi di Messina), Giuseppina Gullifa (Sapienza Università di Roma), Andrea Idili (University of California – Santa Barbara), Anna Illiano (Università degli Studi di Napoli Federico II), Tommaso Lomonaco (Università di Pisa), Laura Montali (Alma Mater Studiorum Università di Bologna), Maria Sole Zalaffi (Università Ca' Foscari Venezia).

The intense social programme brought together all the delegates in a pleasant and stimulating atmosphere, starting from the welcome cocktail on Sunday Sept. 22nd and the Gala dinner on Wednesday Sept. 25th, in the exclusive location of the Sala Zonno on the city promenade. Particularly appreciated were the social tours on Tuesday Sept. 24th (guided tour to Bari down town and Trulli of Alberobello – UNESCO heritage site) that gave the opportunity to get in touch with the food and traditions of Apulia region.

At the end of this challenging and rewarding experience, the local Organizing Committee expresses sincere gratitude to all the delegates and sponsors: Thermo Fisher Scientific, Levanchimica, Merck, PerkinElmer, Shimadzu, EdiSES, Lab Instruments, Nordtest, Lab Service Analytica, Waters, Granoro, Agridè.
Their active participation made the event successful.

Francesco Palmisano & Luigia Sabbatini
Congress Chairs

AIM OF THE CONGRESS

The Congress of the Analytical Chemistry Division of the Italian Chemical Society is a yearly conference covering the most recent achievements in the field of modern analytical methodologies and applications in different thematic areas.

Organized in plenary lectures, key-notes, oral and poster communications, the XXVIII edition covered the following main topics:

Sensors and Biosensors

Separation Sciences

Food and Nutraceuticals

Environment Analytical Chemistry

Cultural Heritage

Analytical Spectroscopy

Forensic Chemistry

Bioanalytics

Omics and Mass Spectrometry

Chemometrics

Solution Equilibria and Speciation

Electroanalytical Chemistry

Material Science

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PROGRAMME

	Sunday 22	Monday 23			Tuesday 24			Wednesday 25			Thursday 26		
08:00		Registration											
08:30													
09:00		Opening Ceremony			PL2			PL3			PL4		
09:30													
10:00		Lectio Magistralis			KN3	KN4	KN5	KN6	KN7	KN8	KN9	KN10	KN11
10:30		Young Researcher Award Lecture (GR)			CHEM1	AS1	SS2	FN1	MAT	SS3	SB3	BIO2	EAC4
11:00		coffee break			coffee break			coffee break			coffee break		
11:30		PL1 + KN1 + KN2			CHEM2	EAC2	SB2	FN2	EAC3	SS4	SB4	BIO3	AS2
12:00													
12:30													
13:00		lunch + PS1			lunch + PS2			lunch + PS3			Closing Ceremony		
13:30													
14:00													
14:30													
15:00		SS1	SB1	CH				EC	SES1	OMS1			
15:30													
16:00					Social Tour								
16:30		coffee break						coffee break					
17:00		EAC1	BIO1	FC				FN3	SES2	OMS2			
17:30													
18:00	Registration & get together party							Divisional groups meeting					
18:30		Invited talk											
19:00		Meeting - Awards and Medals of the Analytical Chemistry Division											
19:30													
20:00													
20:30								Gala dinner					

Legend

PL – Plenary Lectures; **KN** – Keynote Lectures; **PS** – Poster Session; **SS** – Separation Science; **SB** – Sensors and Biosensors; **CH** – Cultural Heritage; **BIO** – Bioanalytics; **EAC** – Environmental Analytical Chemistry; **FC** – Forensic Chemistry; **CHEM** – Chemometrics; **AS** – Analytical Spectroscopy; **FN** – Food and Nutraceuticals; **MAT** – Materials science; **EC** – Electroanalytical Chemistry; **SES** – Solution Equilibria and Speciation; **OMS** – Omics and Mass Spectrometry

Book of Abstracts

Contents

Lectio Magistralis

Young Researcher Award Lecture

Plenary Lectures

Keynote Lectures

Oral presentations

Poster presentations

This ebook contains a collection of the abstracts accepted for presentation at the XXVIII Congress of the Analytical Chemistry Division of the Italian Chemical Society (Bari, 22 – 26 September 2019)

Lectio Magistralis

Lectio Magistralis

HOMO SAPIENS HISTORY AND PERIODIC TABLE OF ELEMENTS: AN INSEPARABLE PAIR

A. Zecchina

Professore Emerito Università degli Studi di Torino, Accademico dei Lincei

In recent times the exploitation of the elements of the periodic table is becoming generalized and is involving additional elements like lithium and rare earths. However, if the growing need of strategic elements and products in the XIX-XX century is considered, this global phenomenon is not looking so exceptional. In this conference the role of periodic table as the basis for the increment of human welfare is described. The problem of the increasing competition for strategic elements and products is also considered.

Young Researcher Award Lecture

GR

PAPER: A NOVEL MULTI-TASKING RESOURCE IN ANALYTICAL CHEMISTRY

S. Cinti

Dipartimento di Farmacia, Università degli Studi di Napoli "Federico II", Napoli, Italy

The growth of (bio)sensors in analytical chemistry is mainly attributable to the possibility of realizing smart platforms, which are affordable, effective, portable and user-friendly. Among the various strategies to develop these devices, the electroanalysis is gaining a leader position in the development and commercialization of analytical devices: it is mainly dependent on the operational simplicity and on the absence of interferences due to colored/turbid solutions (which limits colorimetric tests). Worldwide, blood-glucometer is the most sold example of an easy-to-use device and it represents ca. 90% of the entire biosensors global market [1]. Cooperation of diverse disciplines such as chemistry, biology, material science, and engineering, is pushing electroanalytical methods towards the realization of low-cost, remarkable sensitivity and low-requirement devices. In particular, paper-based devices are always more gaining a relevant position in the field of sensors. The continuous demand for affordable, simple, sustainable, and portable devices, is making paper as the ideal basis towards the realization of analytical tools for the easy self-testing. However, the possibilities around the use of paper are not limited to its use as an alternative substrate in strips fabrication (instead of plastic-based). The active role of paper, in particular the filter one, is extended towards i) the loading of the reagents to make an assay reagent-free, ii) the filtering of the gross impurities in complex matrices, iii) the manufacturing of 2D and 3D architectures, and iv) the synthesis of nanoparticles for sensing application. Many examples that highlight the features of paper-based substrates for sensing applications within the environmental and clinical fields are presented.

Plenary Lectures

PL1

SOLID PHASE MICROEXTRACTION: QUO VADIS?

J. Pawliszyn

Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada

The talk will cover sampling/sample preparation devices developed in my laboratories which facilitate practice of "Green" analytical chemistry. Focus of the presentation will be on recent developments of new morphologies of extracting materials and novel sampling configurations as well as approaches compatible with high throughput lab and/or on-site determinations. The recent development of matrix compatible Solid Phase Microextraction (SPME) coatings lead to interesting features experienced during extraction, some of them not anticipated. They are not limited to elimination of fouling and saturation effects during direct extraction of complex samples, but also balance coverage property, enabling "via free form" clean extraction of small molecules widely varying in physical properties leading to some interesting applications. For example, on-site sampling, in-vivo metabolomics, and rapid screening via direct coupling of sample preparation to mass spectrometry were facilitated by this development. Food, pharmaceutical, clinical and medical application of this chemical biopsy tool for in-vivo monitoring and rapid diagnosis will be emphasized.
Different geometries of SPME will be discussed. One of the more recent approaches developed is thin film solid phase microextraction (TF-SPME). It has been designed for both laboratory and on-site deployment of integrated sampling/enrichment approach at the same time having large sorbent volume forming thin and high surface area coating and therefore facilitating rapid extraction. Various support geometries can be coated with the thin films which is the most appropriate for given application. The practical and fundamental advantages of the SPME technology in aquatic and air sampling investigations will be discussed and demonstrated on practical examples. Both spot and time-weighted average sampling are perfumed using this approach to investigate presence of relevant compounds present in aqueous media and air. The unique features of the microextraction technique facilitate its application for determination of the distribution and characterization of target analytes in real samples not only in laboratory, but also on-site and in-vivo measurements. Number of interesting on-site applications including direct underwater sampling with remotely operated vehicles (ROVs) and sampling and transport of the devices via drones.

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PL2

Metabolomics by numbers: lessons from large-scale phenotyping

R. Goodacre

Department of Biochemistry, Institute of Integrative Biology, University of Liverpool, Liverpool, UK

Metabolomics is a growing discipline that allows the analysis of the thousands of structural different small molecules found within a biological system. These metabolites can be measured using a variety of different analytical approaches and we have developed gas chromatography mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LC-MS) for this purpose [1]. I shall provide an overview of metabolomics and lessons learnt from of our large-scale human serum metabolome project where we profiled 1200 healthy individuals [2]. Using these protocols we then went on to profile another ~1200 ageing individuals and identified key metabolic dysregulation which were drivers behind human frailty, which were validated in a further ~760 ageing individuals [3].

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PL3

THE QUALITY CONTROL OF THE PHARMACEUTICAL INDUSTRY: THE CONNECTION DEPARTMENT BETWEEN THE PRODUCTION OF THE DRUG AND ITS ARRIVAL ON THE MARKET

L. Perani

QP/Quality Manager - Roche Spa, Monza, Italy

The Quality Control Department [1], in the pharmaceutical industry, is undergoing a wide transformation in line with the evolution towards Industry 4.0.
No longer just "data production department" but a deeply interconnected structure with all the corporate structures: the support develops at the level of the entire production cycle of the drug from the control of raw materials, to that of the environments, to the finished product ready to be distributed on the market.
It is a leading role of the "investigations" even among the most intricate (anomalous data, complaints from the market) and is now able to do all this in a "lean" perspective with attention to time and costs.
Finally, it is the department where latest-generation technology, automation and "paperless" concepts lend themselves to being tested and possibly implemented in compliance with the strict regulations in force.
For that reason, the analytical techniques supporting such as complicated department should support fast and repeatable tests, be as much as possible automatable with the respect of budget constraints.

References

[1] EudraLex - Volume 4 - Good Manufacturing Practice (GMP) guidelines

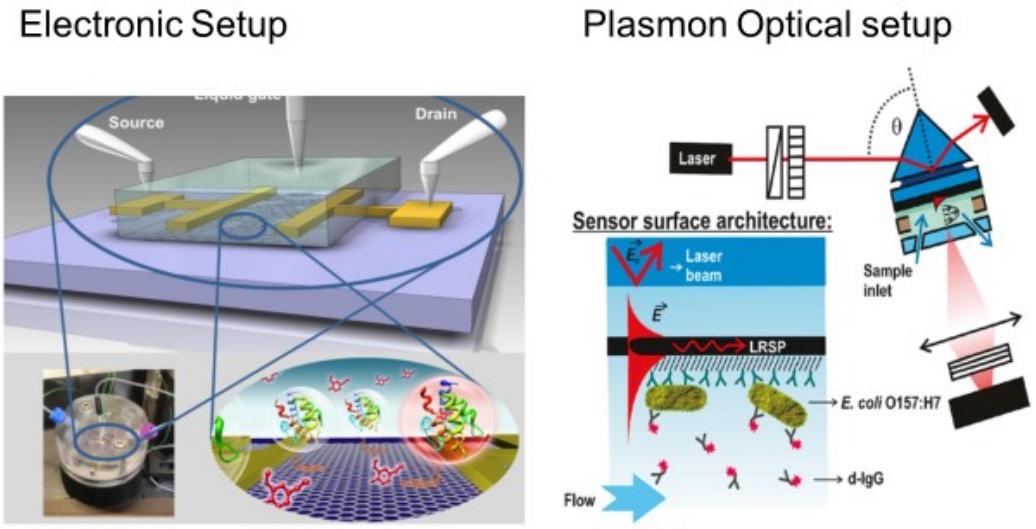
PL4

BIO-SENSING: OPTICAL OR BY ELECTRONICS?

W. Knoll

AIT Austrian Institute of Technology, Vienna, Austria

The race in Protein, DNA, or small molecule diagnostics between optical detection principles (fluorescence, surface plasmons, optical waveguides, etc.) and electrical/ electrochemical/electronic concepts is not decided yet. Both scientific communities continue to offer solutions for fast, multiplexed, simple and cheap detection of peptides, proteins, oligonucleotides, PCR amplicons, small molecules like toxins, odorants, etc. Most likely, the competition will never see a single winner that meets all needs because the different practical formats and boundary conditions for applications, as well as, market requirements may ask for specific and unique solutions that could be better achieved in one case by optics and in another situation by electronics.
Along these lines, we will briefly review the state of the art of both categories of diagnostics and will present a number of examples of what has been demonstrated for the sensitive detection of DNA by monitoring surface hybridization reactions of target strands binding from the analyte solution to surface-attached capture oligonucleotides. Other examples concern the quantitative monitoring of proteins, e.g., antibodies binding directly to the surface-immobilized antigen, or the detection of small analytes, e.g., odorant molecules recognized by odorant binding proteins immobilized on the transducer surface. A particular emphasis will be put on the physico-chemical principles of these surface recognition and binding (or dissociation) reactions in order to be able to develop criteria of how to optimize sensitivity, selectivity, etc.



Keynote Lectures

KN1

FIRST PILOT STUDY OF SELECTED ION FLOW TUBE-MASS SPECTROMETRY (SIFT-MS) IN HERITAGE SCIENCE: CHARACTERIZATION OF NATURAL AND SYNTHETIC PAINT VARNISHES BY PORTABLE MASS SPECTROMETRY

F. Modugno[1], J. La Nasa[1], A. Carretta[2], M.P. Colombini[1], I. Degano[1]

[1]*SCIBEC, Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Italy*
[2]*SRA Instruments, Cernusco sul Naviglio, Milano, Italy*

The identification at molecular level of organic materials in heritage objects as paintings requires in most cases the collection of micro-samples followed by micro-destructive analysis. In this study, we explore for the first time the possibility to characterize natural and synthetic resins used as paint varnishes by mean of non-invasive analysis of released volatile organic compounds (VOCs) through selected ion flow tube-mass spectrometry (SIFT-MS). SIFT-MS is a portable direct mass spectrometric technique that achieves the analysis of VOCs at trace levels in real time, by controlled ultra-soft chemical ionization using different chemical ionization agents. We tested the portable instrumentation on different reference resins used as paint varnishes, both natural (mastic, dammar, and colophony) and synthetic (Paraloid B67, MS2A, Regalrez 1094, and polyvinyl acetate), to evaluate the possibility to obtain qualitative data for the identification of these materials in heritage objects avoiding any sampling. his new analytical approach was validated by comparison with the traditional approach for VOCs analysis based on SPME-GC/MS analysis.

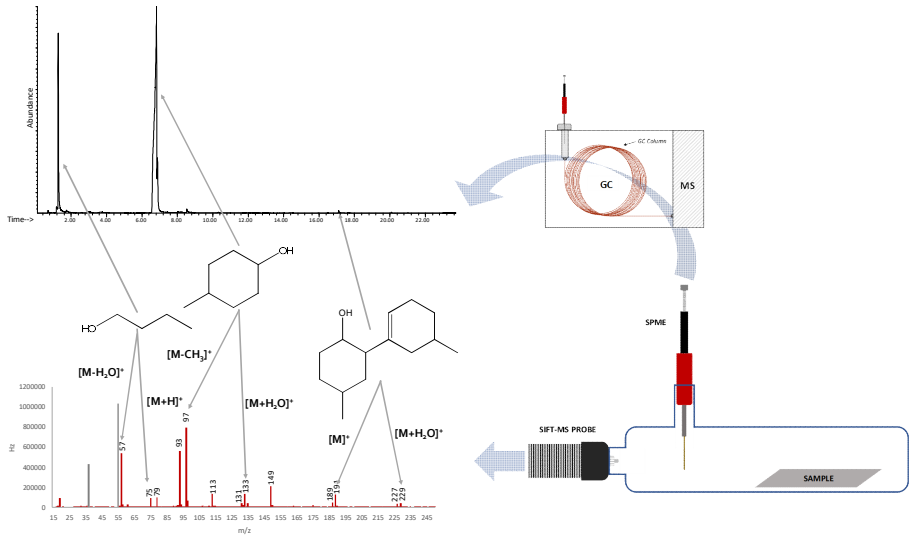


Figure 1. Exposure chamber used for the SPME-GC/MS and SIFT-MS analysis

The promising results obtained in this survey represent the first step in the development of a completely new analytical approach for the non-invasive/non-destructive characterization of

organic materials. The advantages in the use of this instrumentation, such as the low limits of detection, the high selectivity, and the possibility to perform in situ analysis, could be extremely relevant within cultural heritage where the characterization of organic materials requires novel analytical approaches not needing invasive approaches.
The possibility to interface this portable SIFT-MS instrumentation to microclimate frames could be a powerful combination for the characterization of the organic materials through the analysis of the VOC profile. Moreover, the development of exposure chambers with different dimensions suitable for specific artworks or archaeological objects allows a wider range of applications, including the non-invasive identification of archaeological organic residues.

KN2

CHEMILUMINESCENCE LIGHTS UP BIOSENSORS FOR SPACE EXPLORATION

M. Mirasoli[1], M. Zangheri[1], E. Marchegiani[1], A. Porchetta[2], F. Di Nardo[3], P. Simoni[4], L. Anfossi[3], C. Giovannoli[3], A. Nascetti[5], C. Baggiani[3], A. Roda[1]

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[3]*Department of Chemistry, University of Turin, Turin, Italy*
[4]*Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy*
[5]*School of Aerospace Engineering, Sapienza University of Rome, Italy*

Space is attracting more and more interest and great efforts are aimed at achieving unexplored destinations, such as Mars. In particular, the exploration of Mars is scheduled for 2030 and in view of this goal many issues must be solved. Indeed, Mars involves long-term missions, since the journey requires 6 months meaning that the astronauts will have to be trained and equipped for any eventuality. In this context, one of the most important issues is the on-board monitoring of astronauts' health. There is a strong demand for simple portable analytical devices that astronauts can use to perform clinical chemistry analyses during space missions. These devices should be sensitive, allowing quantitative analyses of samples such as saliva or sweat without preanalytical treatment using very simple procedure and they should be unaffected by microgravity. Moreover, they should be light, energy-efficient, and occupy minimal space remaining stable over long-term storage. In addition, they must meet NASA's strict safety requirements for flying payloads.
In recent years we participated to MARS500, a simulated mission to Mars organized by the institute for Biomediacl Problems (IBMP) and by the European Space Agency (ESA) in Moscow. In order to monitor the subjects' gastrointestinal system health status, we developed portable bioassays to evaluate gastrointestinal motility through breath tests and to measure inflammation biomarkers in stools [1].
More recently a real flight experiment was performed onboard the International Space Station (ISS). As part of the IN SITU Bioanalysis project, we designed and developed a device for analysing salivary levels of cortisol, a marker of chronic stress. The device comprises a chemiluminescence (CL) reader, which uses a sensitive cooled CCD camera, and disposable cartridges produced by 3D-printing technology. Cortisol analysis is performed with a CL-based Lateral Flow ImmunoAssay (LFIA), in which the flow of sample and reagents is driven by capillarity. It thus operates in a gravity-independent manner, bypassing the need for pumps [2,3]. The LFIA strip and reagents are located in a sealed microfluidic element, which provides the level of containment according to NASA's safety requirements. This microfluidic element, enclosed in the cartridge, is operated by external buttons and valves. To perform

sensitive quantitative analyses, the device uses enhanced CL imaging based on the horseradish peroxidase (HRP)/luminol/peroxide CL system.
The project, carried out in collaboration with ALTEC SpA (Turin), was financed and coordinated by the Agenzia Spaziale Italiana (ASI), which also provided its own resources aboard the ISS, and supported by Kayser Italia (Livorno) as an ISS control centre, interacting with NASA. The astronaut Paolo Nespoli tested the device onboard the ISS during "VITA" mission (July-December 2017).
Another important aspect for the future missions, is the possibility of analyzing in-situ material samples in search of organic molecules, amino acids, nucleic acids, polysaccharides and other biological systems molecules.
Recently, the increasing development of extremely compact systems relying on microfluidics, commonly known as lab-on-chip devices, has gained much attention thanks to their favorable characteristics in terms of reduced size and weight, very low sample and reagent consumption, reduced analysis time and, often, superior achievable performances in terms of limits-of-detection. Lab-on-chip devices are extremely suitable for space missions. In this context, we report about the design and optimization of new analytical platform for the multiparametric detection of bio-organic molecules outside of the Earth. In particular we optimized a DNA switch based on CL detection for the identification of adenosine triphosphate (ATP) which is an extant life biomarker. The DNA switch was implemented into a portable device, composed of a microfluidic network based on capillary forces for the handling of samples and reagents, a set of functionalized detection sites where the bioassay was carried out and an array of thin-film hydrogenated amorphous silicon (a-Si:H) photosensors for the detection of the analytical CL signal. The implementation of this functional module provided a compact and fully integrated device together with a low power consumption.

Financial contributions from the Italian Space Agency (ASI). We are grateful to the Italian Space Agency (ASI) for making available its resources onboard the ISS.

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KN3

REMARKS ON DATA CORRELATION

R. Todeschini, V. Consonni

Milano Chemometrics and QSAR Research Group, Department of Environmental Sciences, University of Milano-Bicocca, Milano, Italy

The correlation within a multivariate data set may influence the results of several statistical and chemometric methods. For instance, the parameters of regression models, the number of significant principal components in PCA and the composition of optimal informative subsets of objects in experimental design may change on the basis of the level of data correlation.
When using such methods it is thus essential to have a good estimate of how much data variability is related to systematic, and potentially useful, information rather than random noise and chance correlation. Indeed, random noise or chance correlation are always potentially present, making models both unstable and unreliable and giving undesired and sudden bias in data exploration.
In this presentation, some topics related to the concept of multivariate correlation, and specifically how to obtain information from correlated variables and the strategies to control and exploit this information in chemometrics [1-4], will be briefly introduced and discussed.

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KN4

QUARTZ-ENHANCED PHOTOACOUSTIC DETECTION OF HYDROCARBONS FOR OIL EXPLORATION AND ENVIRONMENTAL MONITORING

A. Sampaolo[1], M. Giglio[1], A. Zifarelli[1], G. Menduni[1,2], A. Elefante[1], V. Passaro[2], P. Patimisco[1], F.K. Tittel[3], V. Spagnolo[1]

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[2]*Photonics Research Group, Dipartimento di Ingegneria Elettrica e dell'informazione, Politecnico di Bari, Italy*
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Oil exploration represents a wide research field in which science & technology mainly focus their efforts on two critical tasks: improvement of the drilling sequence efficiency and management of environmental impact. Hydrocarbon detection in the gas phase is a powerful tool for guiding downstream operations as well as providing a constant monitoring of the ambient air around the well site. Indeed, a gas sensor-assisted drilling would avoid an uncountable number of unnecessary holes for dry wells, which results in a huge money saving and a more sustainable impact on the seabed. A swarm of drones equipped with hydrocarbon sensors can be trained to detect and report natural gas leaks along the pipelines to avoid explosions and pollution.
The main advantage in employing laser-based spectroscopic sensors is their capability to target gas species with high selectivity and detection sensitivity. Most of the spectroscopic techniques rely on multi-pass absorption cells or resonant optical cavities to enhance the interaction pathlength between the laser radiation and the absorbing molecules or to boost the available laser power. In both cases, such sensors can be bulky and require the use of optical detectors, which in turn are inadequate for working in harsh environments where the temperature can change sharply.
In this context, we report quartz-enhanced photoacoustic spectroscopy (QEPAS) as a valuable candidate for natural gas analysis in the oil & gas field. The core element of any QEPAS system is the quartz tuning fork, employed as a high quality-factor optoacoustic transducer, capable of operating in a wide range of temperature and pressure [1,2]. The robustness and compactness of these sensors, together with the possibility to avoid the use of optical detectors, represent the basis for the development of a new generation of small-sized gas spectrometers to be potentially i) employed downhole for source rock characterization and estimation of oil & gas reserves, and ii) mounted on drones to constantly monitor the air composition around the well site.

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In this presentation will be given an overlook on shoe-box sized QEPAS systems capable of: i) selective detect methane and ethane in the parts-per billion range, and propane in the parts-per-million range, by employing a single interband cascade laser emitting at 3.345 μm [3]; ii) selective detection of $^{12}CH_4$ and $^{13}CH_4$ isotopes at the parts-per-billion sensitivity level by employing a quantum cascade laser operating around 7.73 μm. Representatives QEPAS spectra measured for methane and ethane are shown in Fig. 1.

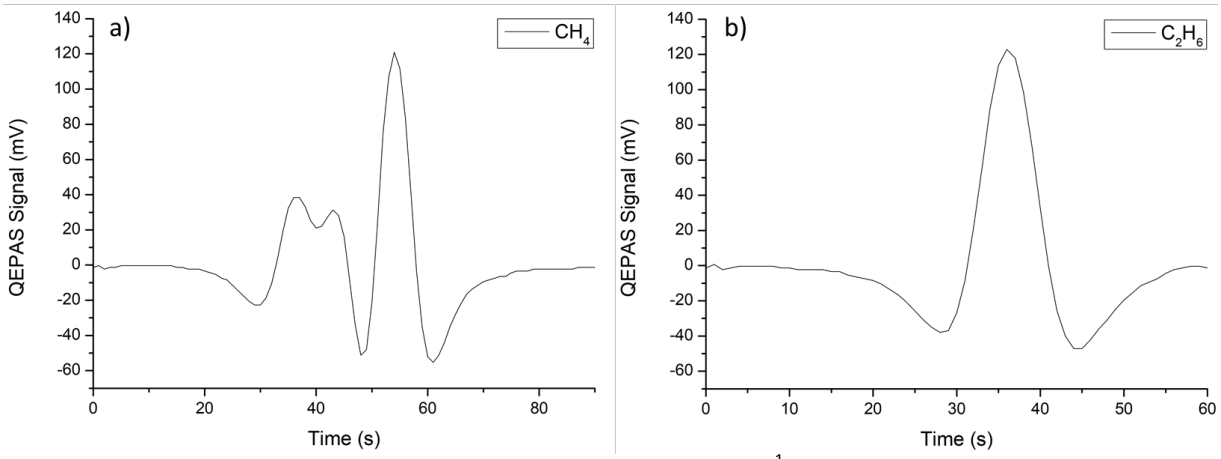

Figure 1. a) QEPAS signal due to methane absorption at 2988.8 cm^{-1} and b) QEPAS signal due to ethane absorption @ 2986.2 cm^{-1} for a mixture of CH_4-900 ppm, C_2H_6-100 ppm in pure N_2 at atmospheric pressure.

An alternative lightweight and low power consumption prototype will be presented as the most compatible with the drone technology state of the art. Two laser diodes beams are combined in a single fiber and its output coupled with the acoustic detection module for simultaneous near-IR detection of methane/ethane or methane/water vapor in the parts-per-million sensitivity level.

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KN5

EXPLOITING THE COMPLEXITY OF FOOD AROMA BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY COUPLED TO TIME OF FLIGHT MASS SPECTROMETRY FEATURING TANDEM IONIZATION

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The process of chemical fingerprinting can substantiate quality differences arising from botanical and geographical origins of primary food ingredients, the impact of post-harvest practices and production processes (traditional *vs.* industrial) as well as shelf-life evolution of finished products. Any analytical platform and/or methodology capable of a comprehensive coverage of chemical traits of food when they are related to the perceived quality –sensory quality, raw material authenticity and processing impact – will contribute to the fundamental step of quality assessment and provide solid foundation for consumer-tailored strategies to improve acceptance and loyalty.
This contribution discusses the strategic role of comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOF MS) featuring tandem ionization and accompanied by pattern recognition using template matching for data processing to unravel the quality traits of high-quality food.
Examples, taken from authors research experience, deal with high-quality cocoa, a food commodity of global economic interest, and extra-virgin olive oil, a local commodity of strategic relevance for the Mediterranean producing countries (Spain, Italy, Greece, Tunisia). Both commodities have an intrinsic added value related to their flavor profiles and perceived qualities, that are 80-90% due to aroma-active compounds. Likewise, the origin and processing signatures are of great help in driving industrial strategies in products reformulation and blending.

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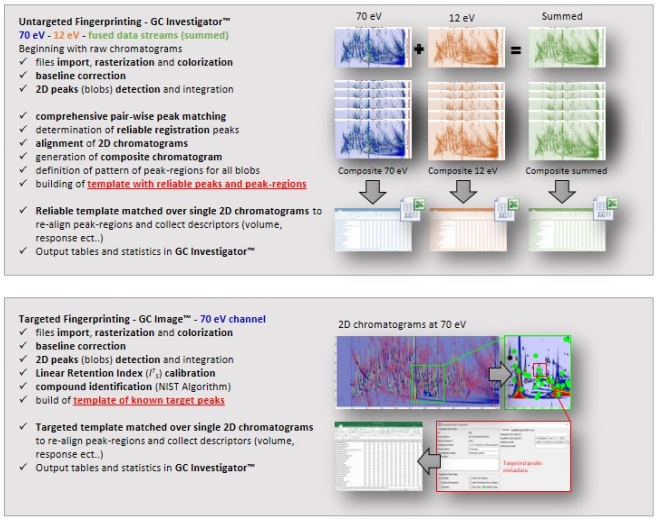

Figure 1. Tandem ionization data exploration by untargeted/targeted *UT fingerprinting*

The approach known as un-targeted/targeted fingerprinting (i.e., *UT fingerprinting*) based on single and tandem ionization data streams is explored and tandem signals examined for their information potential (schematic illustration in Figure 1) in terms of products aroma, technological signature and geographical origin peculiarities.

The advantages of a true multidimensionality, from sample preparation (the zeroth dimension of an analytical system) to separation (GC×GC) and analyte detection and identification (TOF MS and tandem ionization) are discussed also in the perspective of a modern approach to food quality assessment where analytical chemistry would play a pivotal role.

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KN6

RP-HPLC PHENOLIC COMPOUNDS PROFILE OF OLIVE LEAVES, EXTRA VIRGIN OLIVE OIL AND OLIVE MILL WASTE WATER FROM MOROCCO USING A DESIGN OF EXPERIMENTS (DoE) APPROACH

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Phenolic compounds are a large class of plant secondary metabolites comprising a great number of heterogeneous structures that range from simple molecules to highly polymerized compounds, which are commonly bound to other molecules, frequently to sugars, although phenolic compounds in free form also occur in plant tissues. These compounds form an integral part of human diet, contributing to the sensory properties of plant-based aliments and to their beneficial effects on human health. The phenolic compounds, as well as many other plant secondary metabolites, have also a remarkable position as bioactive components in medicinal plants and have evidenced to exhibit numerous biological activities and a variety of health benefits against chronic and degenerative human diseases. A variety of instrumental analytical separation techniques are employed to identify and quantify each of the main phenolic compounds occurring in plants and plant-derived food products. Among them, high performance liquid chromatography, mostly in reversed phase separation mode (RP-HPLC), is the techniques of choice [1]. This communication discusses the results of a study carried out to investigate a variety of factors that influence the RP-HPLC separation of phenolic compounds occurring in olive leaves, extra virgin olive oil (EVOO) and oil mill waste water (OMWW). The study has been carried out in the framework of an EU Project (OliveNet 734899) aimed at the valorization and marketing of new products based on bioactive compounds from Olea europaea, with particular attention to phenolic compounds.

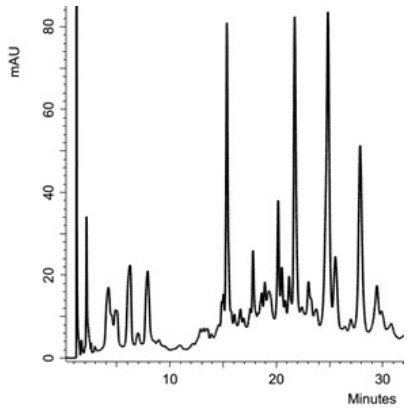

Figure 1. RP-HPLC separation of phenolic compounds extracted from leaves of Olea europaea, variety Koroneiki.

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After the processing of olives for olive oil extraction, less than 1–2% of the phenolic compounds are found in EVOO, whereas the majority are lost either in the pomace or in the OMWW. Significant content of phenolic compounds are also occurring in the leaves of the Olea europaea trees, which form a huge bulk of solid wastes produced by the cultivation of olive trees (see Figure 1). Therefore, both the olive processing industry and the cultivation of olive trees are responsible for the production of large quantities of by-products, which might either create major environmental problems when are not correctly disposed in nature or become a source of novel active ingredients that could be used in the pharmaceutical, nutraceutical and cosmetic industry.

This presentation evaluates the influence of pH and mobile phase composition, column temperature and gradient elution program on the RP-HPLC separation of phenolic compounds occurring in olive leaves, EVOO and OMWW. Appropriate selection of the mobile phase in RP-HPLC involves the evaluation of the equilibrium in solution that might take place between the analytes and the components of the liquid phase. The ionogenic nature of most of the considered compounds requires the control of the protonic equilibrium in solution, which is performed by incorporating proper additives into the mobile phase, such as an organic acid or a buffer. The constituents of the mobile phase do not limit their action at controlling the protonic equilibrium. They also might interact with the analytes, for examples by an ion-pairing mechanism, with the result of altering their chromatographic retention, which is modulated by the chemical composition and concentration of the organic solvent, which progressively increases during the analysis in gradient elution mode.

In this study, the appropriate selection of the separation conditions employed in RP-HPLC has been conducted by a Design of Experiments (DoE) approach, which has allowed the simultaneous optimization of pH and composition of the hydro-organic mobile phase, shape and duration of the binary gradient elution program, and column temperature on the basis of the retention times and peak areas of the analytes of interest, obtained by a limited number of experiments. The presentation describes the use of the above experimental data to construct 3-D resolution maps, which have been used to evaluate the influence of column temperature, duration and shape of the elution gradient, pH and composition of the mobile phase on the retention and resolution of a variety of analytes. In our study, 3-D resolution maps were constructed using either mixtures of standard phenolic compounds or samples of these compounds extracted from EVOO, OMWW and olive lives. The goal of our study was to model the variations that can help for the better selection of the mobile phase composition and gradient elution mode, in order to improve peak resolution and to reduce the analysis time, using a limited number of experiments and, consequently, reduced amounts of expensive and environmentally harmful chemicals. Excellent correlation between simulated and experimental separations of phenolic compounds are demonstrated. The application of the above DoE approach to study the phenolic compounds profile of EVOO, OMWW and olive lives from Morocco is illustrated and discussed.

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KN7

ELECTRODEPOSITION AND SURFACE ANALYSIS FOR NEW MATERIALS FOR ENERGY APPLICATIONS

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The global environmental concerns and the escalating demand for energy, coupled with a steady progress in renewable energy technologies, are opening up new opportunities for the utilization of renewable energy resources. 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. These combined experiments have recently been widely used for the study of new-generation solar cells and low-precious-content fuel cells up to the preparation of sensors for Industry4.0. In the field of thin-film solar cells electrodeposition is well known for depositing metals and metallic alloys at the industrial level, with a wide range of applications from large area surface treatments to most advanced electronic industries. Electrodeposition of semiconducting materials represents a new challenge, not only from the academic point of view, but also from the economic point of view, since this method presents interesting characteristics for large area, low cost and generally low temperature and soft processing of materials.

In this presentation, we provide an overview of different cases of study where basic electrochemical techniques have been combined with spectroscopic, microscopic and structural techniques is crucial for characterizing the structure-activity relationship for many different Materials for Energy Applications [1].

In one case of study, we exploited alternated metal electrodepositions by E-ALD (Electrochemical Atomic Layer Deposition) to obtain multilayered thin films. E-ALD provided a tight control over the materials' growth, at the nanometer level, even when an entire p-n junction was deposited. We performed a thorough structural study of these composite ultra-thin films by means of electrochemical operando SXRD (Surface X-Ray Diffraction) and FEXRAV [2] (Fixed Energy X-Ray Absorption Voltammetry) experiments performed at ID03 in Grenoble [3].

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In the field of fuel cells, we aimed at reducing the quantity of catalytic material in order to meet the industrial demand for alternative catalysts be used in place of Platinum-group metals [4]. To remove and replace platinum with less expensive materials, we exploited a synergistic mechanism involving one metal able to break the O-O bond of molecular oxygen and second metal species capable to reduce the resulting adsorbed atomic oxygen. The presence of specific metals together with a high carbon content are essential requirements for catalysts for Oxygen Reduction Reaction (ORR [5]).

Furthermore, we developed and characterized new catalyst for fuel cells, based on automotive tires recycling [6]. There are several technologies for tires recycling, including pyrolysis: a thermal decomposition process performed at higher temperature in an inert atmosphere which allows the transformation of complex substances in simple molecules. We worked at modified surfaces implementing novel electrodeposited catalysts obtained from the microwave assisted pyrolysis (MAP) of waste tires, and we proposed them for direct alcohol fuel cells [7].

In conclusion, we believe that one the fundamental aims of analytical chemistry applied to surface sciences is to reckon the relationship between the properties of a device, and the morphological and structural characteristics of the surface itself.

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KN8

ON-LINE COUPLED LIQUID CHROMATOGRAPHY – COMPREHENSIVE TWO DIMENSIONAL GAS CHROMATOGRAPHY WITH DUAL DETECTION FOR THE ANALYSIS OF MINERAL OIL IN COSMETICS

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An on-line liquid chromatography-comprehensive two-dimensional gas chromatography (LC-GC×GC) separation process, combined with a dual detection system, namely triple quadrupole mass spectrometry and flame ionization detection (FID), was developed for the analysis of cosmetic lip care products.
Moreover, in the present contribution, a lab-developed LC-GC interface will be presented, employing two six-port two-position valves and a modified programmed temperature vaporizing (PTV) injector. The PTV injector has been modified in order to improve the recovery of low boiling compounds.
The LC step was carried out by using a silica column, with this enabling the separation of mineral oil saturated hydrocarbons (MOSH), as well as polyolefin oligomeric saturated hydrocarbons (POSH), from the mineral oil aromatic hydrocarbon (MOAH) families. Each chemical class was then on-line subjected to GC×GC-MS/FID analysis, using a medium polarity-low polarity column combination.
Notwithstanding the utility of the flame ionization detector for quantification purposes, it is obviously also desirable to obtain information on the type of hydrocarbons present (of mineral or synthetic origin), in order to identify a potential contamination source.
Following method optimization, various analytical figures of merit (method linearity, intra- and inter-day repeatability, limits of detection and quantification, and injector discrimination) were measured. The proposed method, enables the detailed qualitative and quantitative analysis of saturated and aromatic hydrocarbons, in a single run and in a fully-automated manner.

KN9

ENGINEERING DNA-NANODEVICES FOR THE RAPID, SINGLE STEP DETECTION OF CLINICALLY RELEVANT ANTIBODIES

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DNA has the most predictable and programmable interactions of any natural or synthetic molecule. It shows unique binding specificity and thermodynamic stability, and it can be rapidly synthesized and modified using automated methods [1]. These features have revealed the unprecedented power in using DNA as a programmable material at the nanoscale. Recent advances in the field have demonstrated the unprecedented capability in using engineered nucleic acid nanodevices for various applications, including biosensing and molecular diagnostics [2].
In this talk we will discuss different strategies to develop optical and electrochemical DNA-nanodevices for the rapid, single-step detection of clinically relevant antibodies. Antibody detection plays a pivotal role in the diagnosis of pathogens and monitoring the success of vaccine immunization. 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. 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).
Motivated by the above arguments, our journey through this topic will start with an optical nucleic acid-based platforms able to measure Immunoglobulins of type G and E (IgG and IgE) levels directly in blood serum and other bodily fluids in few minutes and without washing steps [3, 4]. Our sensing strategies couple the advantages of target-binding induced co-localization and nucleic acid conformational-change nanoswitches. These platforms can be adapted to the detection of any antibody for which the recognition element (i.e. antigen) can be coupled to the nucleic acid anchoring strand. In the second part of the talk we will explore the translation of the optical sensing platform into an electrochemical format which allows both POC applications and large-scale analysis. Finally, we will discuss some clinically relevant applications, such as the monitoring of monoclonal antibody titers (i.e. Trastuzumab) in plasma samples of patients under breast cancer immunotherapy, and the immune response in HIV-infected patients under vaccine treatments.

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KN10

DETERMINATION OF OXIDATIVE STRESS MARKERS IN BIOLOGICAL MATRICES BY MEANS OF UHPLC-MS/MS

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Oxidative stress (OS) is a physiological state leading to Reactive Oxygen Species (ROS) that are negative factor for human's health. OS is involved in aging processes and in many cardiovascular diseases, diabetes, and carcinogenicity processes. It is therefore evident that the need for reliable (bio-)markers for the identification of this physiological state is fundamental. Recently the class of isoprostanes of have been identified as gold standard for *in viv*o OS evaluation, thanks to their robust correlation in lipid peroxidation induced by the presence of ROS. Also other classes of molecules may be used as markers as they provide different information on the OS even in peripheral phenomena or by specific external threats towards the organism (oxysterols, resolvins).
The aim of this research was the development of tailored analytical methods for the evaluation of oxidative stress through the monitoring of specific markers, in order to evaluate the modifications in the physiological state by different point of view. The developed analytical tools may help to evaluate the effect on the oxidative state in humans or model organisms resulting from the exposure of xenobiotics or due to incorrect dietary regimes. The main goal was the development of a reliable analytical method for the determination of the levels of isoprostanes in the urine by means of dispersive Liquid-Liquid Micro Extraction (dLLME), subsequent micro Solid Phase Extraction (μSPE) clean-up and UHPLC-MS/MS analysis. The analytical targets were mainly high sensitivity and effective clean-up of the sample, for the quantification of 8-isoprotaglandine F2α (8-iso-PGF2α) and 5 isoprostane F2α (5-IPF2α), together with some metabolites and/or isomers.
Another goal was the identification and quantification of membrane oxysterols in different matrices, mainly swine and kelp spermatozoa. The presented method is focused mainly on the following oxidized forms: 20d-hydroxycholesterol, 25-hydroxycholesterol, 25-hydroxycholesterol, 22-hydroxycholesterol, 7α- and 7β-hydroxycholesterol and desmosterol. The samples deriving from spermatozoa were processed through a Liquid-Liquid Extraction (LLE) and subsequent clean-up by μSPE.
Some interesting results were also obtained for oxysterols in Zebrafish, as it was established as a model organism in studies of genetics, developmental biology, neurophysiology and biomedicine [1,2]. It is used as a biomarker of the effects of toxins and pollutants in the environment and in the study of the pathogenesis of some human diseases [3]. For Zebrafish embryos the samples were processed by LLE in an orbital shaker; after the withdrawing and drying of organic portion a μSPE clean-up step was performed.

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In addition also studies on resolvins in plasma were carried out as they are involved in inflammatory processes. This group of molecules arise from the n-3 fatty acid docosahexaenoic acid (DHA) or from the long-chain n-3 fatty acid eicosapentaenoic acid (EPA). These mediators, acting via G-coupled protein receptors, have potent anti-inflammatory and proresolving actions [5].
In the case of isoprostanes we must deal with compounds present in very small concentration in the urine; furthermore, they are a challenge for UHPLC-MS/MS analysis due to the relevant matrix effect [7]. Given this problem, we opted for a dLLME extraction which allows a significant enrichment factor of the analytes and a µSPE clean-up. For oxysterols, a different approach was applied, in fact they are not so prone to matrix effect as Isoprostanes and the requested LOQs are significantly higher. They, on the other hand, still face challenges: it was therefore difficult to completely and rapidly disrupt the membrane phospholipids, which was performed by an immersion probe followed by an LLE extraction and then a µSPE clean up to enrich the extract. The chromatographic separation was crucial as all analytes are positional isomers or epimers. Another difficulty of these compounds is their low sensitivity in mass spectrometry without a derivatization process [8]. This step was avoided by working on the extraction method and using an APCI source. For resolvins in plasma dLLME and µSPE were used for extraction and clean-up, while ESI negative ionization was applied for UHPLC-MS/MS analysis.
The obtained results for the different classes of compounds were satisfactory, in fact it was possible to obtain a good extraction with the dLLME (or LLE) technique, succeeded in concentrating the sample and then performing a µSPE clean-up which led to a lower matrix effect and good enrichment factors.

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HUMIC ACID COATED MAGNETIC PARTICLES AS HIGHLY EFFICIENT HETEROGENEOUS PHOTO-FENTON MATERIALS FOR WASTEWATER TREATMENTS

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Contaminants of emerging concern (CECs) are increasingly being detected at low levels in surface water, groundwater and drinking water [1]. Despite their low concentration there is a real concern on their impact on aquatic life and human health due to their toxicity [2]. As a consequence of their incomplete removal in the traditional wastewater treatment plants (WWTPs), considerable efforts have been devoted to develop purification methods able to destroy these contaminants. Advanced oxidation processes (AOPs) have been attracting wide attention, since they are efficient in the degradation/mineralization of organic contaminants through the production of oxidizing species (mainly hydroxyl radicals, $^{\bullet}OH$) [3]. Among AOPs there is increasing interest in Fenton, photo-Fenton and Fenton-like processes that can generate highly reactive species [4] through the reaction between recyclable iron and H_2O_2 or other alternative oxidants (e.g. $S_2O_8^{2-}$). Heterogeneous photo-Fenton reaction with iron-based materials has been proposed as a promising alternative to the homogeneous process due to the easy recovery of the catalyst. In particular, different iron-based materials (morphologically controlled hematite, nanometric magnetite, passivated zero valent iron) has been tested in the recent years with the aim to give insights into the mechanism of transformation of CECs at the surface of these catalysts in Fenton or Fenton-like conditions. [5,6,7] In this light, the analysis of the catalyst composition (e.g. speciation of the active species) and of the species release in solution plays a crucial role. In this work, magnetic particles (MPs) coated with different amount of humic acid (HA), synthesized by co-precipitation method under anoxic and oxygenated conditions, were characterized by different techniques (XPS, XRD spectroscopy, TGA, SEM and FTIR). The ability of these materials to promote heterogeneous Fenton- and photo-Fenton-like processes was investigated using 4-chlorophenol as target pollutant. The HA coating induced an enhancement on the catalyst efficiency both in the dark and under irradiation, showing the best performance at pH below 4 under simulated sunlight irradiation. The comparison between the XPS experimental data (Figure 1) and the observed reactivity suggested an active role of the more defective iron species at the surface which could promote a higher photo-dissolution with an increment of the reactive iron, both at the surface and in solution. Even if the iron ions leached in solution could have a remarkable influence on the oxidation process, a relevant role can be imputed to processes at the solid/liquid interface (heterogeneous reactivity).

KN11

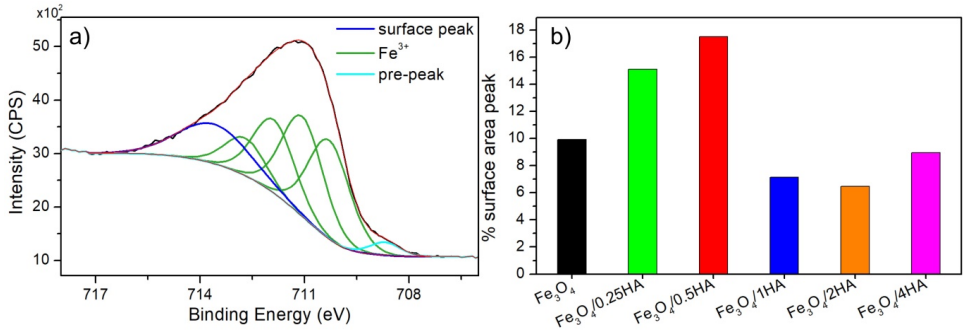

Figure 1. High-resolution spectrum of Fe 2p region of Fe_3O_4/0.5HA (MPs synthesized with HA solution 0.5%) prepared under anoxic conditions (a). Spectrum is charging corrected. Percentage of the surface peak in all samples prepared in anoxic conditions (b).

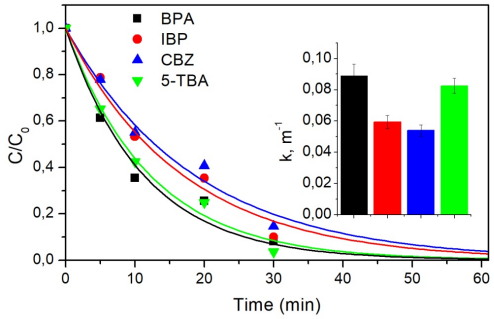

Figure 2. Photo-Fenton-like degradation of CEC mixture (20 µM) in real wastewater. Conditions: pH 3, H_2O_2 1.0 mM, Fe_3O_4/0.5HA 100 mg dm^{-3} (MPs synthesized with HA solution 0.5%). Inset: first-order kinetic constants of the process

The best performing MPs/HA (Fe_3O_4/0.5HA) showed high efficiency for the abatement of CECs, namely Carbamazepine, Ibuprofen, Bisphenol A and 5-Tolylbenzotriazole also in real wastewater (Figure 2) demonstrating that this material can be successfully employed in advanced tertiary treatments for the removal of CECs from urban wastewater by employing cheap and environmental friendly materials and reagents (e.g. H_2O_2) and activating the process through the widely diffused and inexpensive solar irradiation.

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Oral presentations

Invited talk

EUROPEAN PROTEOMICS INFRASTRUCTURE CONSORTIUM - PROVIDING ACCESS (EPIC-XS): A COMMON EUROPEAN PROTEOMICS INITIATIVE

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Protein analysis is essential for understanding the complexity of the communication process that takes place within every living organism. If we are ever going to make significant advancements not only within medical science but also across the life science arena as a whole, we need to fully understand the proteome, to unravel its secrets, to understand how the proteome is controlled, expressed and regulated [1]. Recent successes illustrate the role of mass spectrometry-based proteomics as an indispensable tool for molecular and cellular biology. MS based proteomics has also become an integral part of cancer research, in monitoring the drug response of tumors, understanding mechanisms that lead to cancer pathogenesis, and the design of novel therapeutics. This technology is by no means limited to the medical and diagnostic sectors: the agricultural sector can also attribute advancements in the evolution of novel designer crops to mass spectrometry technology. Furthermore, developments within the proteomics arena has led to the discovery of proteins hidden away not only within flora and fauna but also within other life forms such as tiny microalgae. The research team led by professor Albert Heck at Utrecht University have discovered a highly efficient light harvesting system within this tiny life form which actually hold the secret for the next generation of solar panels [2]. Pioneering research in high-throughput mass spectrometry based proteomics requires state of the art technology, in-house technical know-how, sustainable and robust workflow practices, successful and correct data interpretation and management. The EPIC-XS [3] partnership brings together eighteen European member states spanning fourteen countries, unified in their objective of providing proteomics expertise and mass spectrometry technology to all researchers within the life science arena. The development, expansion and sustainability of proteomics through this initiative will be achieved by providing over 2,400 days of access to state of the art proteomics technologies across eleven access sites and will also provide access to various workshops and training courses. The initiative is funded as part of the European Union's Horizon 2020 work program and is coordinated by professor Albert Heck.

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O1 SS1

DETERMINATION OF VOLATILE COMPOUNDS IN CANNABIS SATIVA L. USING ELECTRONIC NOSE BASED ON PEPTIDE AND HAIRPIN DNA Vs. SOLID-PHASE MICROEXTRACTION AND GC-MS

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Cannabis plants (*Cannabis sativa L.*) produce and accumulate a resin rich in terpenes in glandular trichomes, which are abundant on the surface of the female inflorescence. The volatile constituents of Cannabis have been studied because they represent a potential for fingerprints of different cultivars. Terpene molecules consist of the union of isoprene units in a "head to tail" configuration. The structure of the terpenes can be cyclic or open and may include double bonds, a hydroxyl, a carbonyl or another functional group. Several monoterpenes and sesquiterpenes are important components of cannabis resin as they define some of the unique organoleptic properties, influencing the qualities of different cannabis strains and varieties [1; 2]. Some terpenes, (mono and sesquiterpenes), are very important because they can be responsible for the anti-inflammatory activity (such as beta-caryophyllene which is the predominant sesquiterpene), or pinene (monoterpene) which has been reported as an acetylcholinesterase inhibitor which helps the memory inhibitor that helps memory. Terpenes have high vapor pressures, are extremely volatile and therefore are excellent candidates for analysis performed at the electronic nose or gas chromatography-mass spectrometry [3].

The aim of the work was to develop a methodology based on ZnO-peptide and hp-DNA based QCMs array of gas sensors for the characterization and fingerprint identification of various commercial hemp samples from different Italian companies. Samples have been ground and analyzed with the gas sensor array and a gas chromatography -MS SPME method in parallel.

The data set obtained were studied with multivariate analysis, mainly with the discriminant analysis (PLS-DA). As from literature, the most significant volatile compounds present in the hemp samples were: α-pinene, β-pinene, β-myrcene, D-limonene, linalool, α-terpineol and terpinolene [3]. However, all the volatile components that were identified, were used for the discrimination of the different samples coming from the different Italian companies.

The results obtained showed that the set of sensors based on peptides and hairpin DNA functionalized with nanoparticles allowed a good discrimination between the companies; the percentages of confusion matrix was around 80%. These results were confirmed with gas chromatography-MS SPME analysis.

O1 SS1

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O2 SS1

DEVELOPMENT OF A NEW ANALYTICAL STRATEGY FOR THE SELECTIVE ENRICHMENT OF PHOSPHO-TYROSINE-PEPTIDES BY MOLECULARLY IMPRINTED POLYMERS

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Phosphopeptides derive from the phosphorylation of proteins, which is a very important post-translational modification which regulates the biological activity of proteins. Phosphorylation is a reversible modification catalyzed by kinase enzymes and consists in the covalent bond of a phosphate group to residues of serine, threonine and tyrosine. In this context, tyrosine phosphorylations are very important because tyrosine phosphopeptides (pTyr) could also be used as biomarkers for cancer diagnostics and therapeutics, so identification and determination of these phosphopeptides could provide abundant information for cancer diagnosis. [1] The abundance of pTyr is generally very low, so it is difficult to analyze them with the common preconcentration techniques used for conventional phosphoproteomics analysis [2; 3]. Therefore, it is urgently necessary to develop sorbents for selective enrichment of pTyr with high efficiency. Molecular imprinting technology is a strategy to create polymers with molecular imprints (MIP) i.e. tailored binding sites that can recognize the target. In this work, phosphonate-imprinted magnetic nanoparticles were manufactured using phenylphosphonic acid as a model to mimic the pTyr "epitope" and subsequently used to enrich pTyr with high efficiency and selectivity. Three types of MIPs have been synthesized: in the first MIO material, magnetic nanoparticles (Fe3O4) have been functionalized with TiO_2 and then with a phenylphosphonic acid imprinted silica shell to obtain MagNP sorbents. The second material was synthesized by the same process but had a graphitized carbon black (GCB) core. Finally, the third material was synthesized substituting the TiO2 layer with a urea-based recognition element inspired by our previous reports on neutral receptors for phosphopeptide capture.[4;5]We anticipated that a functional monomer, derived from the reaction of para-amino benzoic acid and 3,5-bis-(trifluoromethyl)-phenyl isocyanate would form strong cyclic hydrogen-bonds with oxyanions such as phosphates. The choice of a urea-based receptor imprinted against different phosphorylated residues can be programmed by selecting different templates. The MIPs decrease the enrichment time and improve the absorption efficiency for pTyr, and the epitope imprinting films give an excellent selective recognition capability to the target. The results indicate that the MIP can enrich the pTyr from the tryptic digest of β-casein and

bovine albumin serum samples with high specificity. The MIPs sorbents show great potential for analysis of the phosphorylation of peptides in complex biological samples.

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O3 SS1

NANOCONFINED ARRAY LIQUID-SOLID MICROEXTRACTION BASED ON CARBON NANOFIBERS

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Among different existing extraction methods, microextraction techniques (METs) are considered nowadays very interesting due to their innovative characteristics[1–3]. Their use in fact allows different benefits, such as a minimum (or null) use of organic solvents, one-step sampling, preconcentration and clean-up procedure, simple operation, on-site derivatization, suitability for on-site, in-site and in-vivo determinations, and easy hyphenation with existing analytical techniques. An impressive number of METs have been set up, especially in the last five years[4]: they can be subdivided in two categories, namely "sample stir" and "flow-through" METs[5]. Solid phase microextraction (SPME) belongs to "sample stir" category and it is the first and still one of the most used MET. New sorbent materials have been employed in order to satisfy different requirements, i.e. specificity, biocompatibility, high recoveries, fast equilibrium time, high sensitivity or reproducibility, reusability, and high anti-fouling properties[5–9]. The type of coating used in the SPME fiber plays then a crucial role in the extraction and desorption processes, since their efficiency will depend on the distribution constant between the analytes and the stationary phase, and then fiber selectivity towards specific analytes in complex matrices. In this work, an innovative and versatile microextraction technique, based on solvent entrapment in carbon nanofibers pores, has been conceived, realised and optimised. The choice of the nanoconfined solvent (NCS) confers to this device a high versatility: it can extract polar, medium polar and/or nonpolar substances from complex matrices. The so-called nanoconfined array liquid-solid microextraction (NALSM) showed excellent extraction recoveries, short extraction time (1 min), high reliability, versatility, and reusability. Carbon nanofibers have been inserted in an umbrella-type device that guarantees a safe insertion in real samples, and the creation of a constant volume liquid zone, that allows on-site, in-site and in-vivo extraction. Due to its versatility, chemical stability and mechanical flexibility, NALSM can be considered a powerful candidate for high-throughput analyses from in-vivo biological samples.

O3 SS1

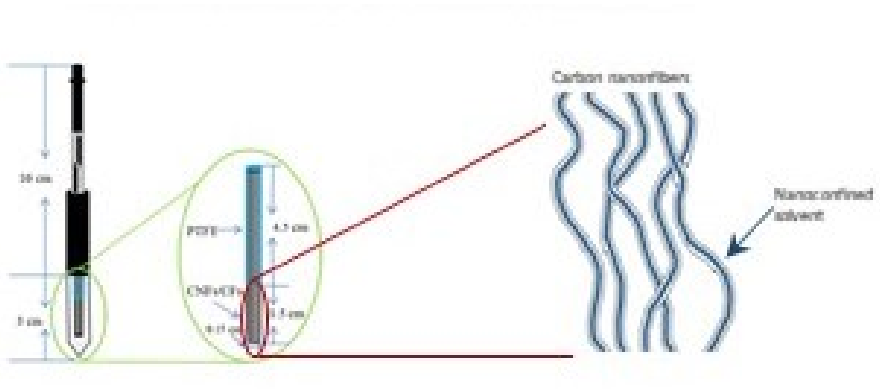

Figure 1. Nanoconfined array liquid-solid microextraction device

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O4 SS1

THERMALLY CONDENSED HUMIC ACIDS ONTO SILICA AS MIXED-MODE SORBENT FOR MULTICLASS EXTRACTION, CLEANUP AND PRE-CONCENTRATION OF STEROIDS IN HUMAN PLASMA

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In this work, a new, straightforward analytical method has been developed for multiclass determination of steroids (progestins, oestrogens, androgens and glucocorticoids) in human plasma. The key part of the procedure is the sample pre-treatment step, that is based on solid-phase extraction (SPE) using a newly proposed carbon-based sorbent able to retain the target compounds from plasma while excluding most of the matrix proteins, which represent serious interferences in the routinely used chromatographic analysis.
The sorbent is obtained by pyrolysis of commercial humic acids (10 wt%) onto micrometric silica [1]. This preparation yields a supported carbonaceous phase (HA-C@silica, carbon phase 1.9 wt%) characterized by an aromatic structure embedding polar oxygenated groups, thus a hydrophilic-lipophilic balanced sorbent able to give multi-type interaction with the solutes. Interestingly, HA-C@silica is able to exclude about 96% protein, similarly to the restricted access material based on modified carbon nanotubes (RACNTs), specifically designed for cleanup of protein samples [2].
The explorative part of the work was carried out on bovine serum albumin (BSA) solutions (phosphate buffer pH 7) to investigate the feasibility of extraction/cleanup of the analytes in a protein-rich matrix. For the preliminary tests, undertaken in 10 g/L BSA solutions using 50 mg sorbent, progesterone, 17β-estradiol, testosterone and cortisone were selected as representative steroids of the four classes. Results indicated full adsorption and elution (using 2 mL methanol), with recovery from 84 to 100% (RSD < 7%, *n*=3) depending on the analyte. The extraction was instead unsatisfactory on the RACNTs, which provided recovery in the range 58-91%, with a larger volume of eluting solvent, reasonably due to a very strong CNT-solute interaction. Control tests on bare silica (pyrolyzed with no humic acids) highlighted the major role of the silica-supported carbons in terms of retention.
The SPE conditions on HA-C@silica were then optimized by a chemometric approach (2^3 factorial design) considering sorbent amount, sample volume and protein concentration as the variables involved in the process. Recovery tests were done at 2 µg/mL spikes. Results clearly indicated a full adsorption working with 100 mg HA-C@silica and 2 mL sample, at a protein concentration of 10 g/L. After extraction, analytes were quantitatively eluted by 2 mL methanol, prior to LC-UV analysis. Good precision was observed, with RSD ≤ 12% (*n*=3). Subsequent trials at lower concentrations (0.2 µg/mL) evidenced that recovery was quantitative but for a complete desorption of progesterone the mixture methanol-acetonitrile (1:1) was required. To improve both cleanup and enrichment factor, several

washings were tested after extraction to remove the small fraction of retained protein, and smaller volumes of eluting solution were applied. It was found that washing with 2% v/v formic acid followed by 30% v/v MeOH (2 mL each) provided an improved cleanup (about 0.4% residual protein in the eluate, Bradford assay); at the same time, elution with 0.5 mL gave enrichment and reduced consumption of organic solvents. The SPE procedure was extended to 15 steroids and recoveries in the range 100-120% were observed. The optimized protocol was applied to human plasma spiked with 2-8 µg/mL before 1:8 dilution, with unchanged recovery. Basing on these findings, SPE experiments at lower concentrations (20-200 ng/mL) close to the steroids levels commonly detectable in human plasma are in progress using LC-MS/MS quantification (MRM mode).
The method avoids ultracentrifugation and protein precipitation, which often are required before extraction/cleanup, and basing on these results it is expected to be a useful tool for routine bio-medical analyses.

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O5 SS1

NANOMIPS BY SOLID PHASE POLYMERIZATION SYNTHESIS: AN INNOVATIVE APPROACH TOWARDS ARTIFICIAL ANTIBODIES FOR ANALYTICAL APPLICATIONS

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Molecularly imprinted polymers (MIPs) are synthetic materials obtained by polymerization in presence of a template target molecule and able to rebind selectively to this target. MIPs in the form of nanoparticles (nanoMIPs) offer good control of the quality of binding sites and morphology of the polymer. Thus, nanoMIPs have the potential to be low-cost and robust alternatives to antibodies in applications as immunoassay, sensoristics and complex sample purification by affinity chromatography. Anyhow, when prepared by traditional synthetic methods, nanoMIPs usefulness has been limited by the presence of residual template and large-scale manufacturing costly, labor intensive and difficult to standardize.

To overcome such limitations a solid-phase polymerization synthesis (SPPS) approach has been proposed recently [1]. It relies on the covalent immobilization of the template onto the surface of a solid support, the fast polymerization of nanoMIPs around the template and the release of the imprinted nanoparticles by changing the medium conditions. The obtained nanoMIPs are virtually free of template and demonstrate high affinity for the target molecule. Moreover, because of an affinity separation step performed on the solid phase after polymerization, poor binders and unproductive polymer are removed, so the final product has more uniform binding characteristics.

Here we present the results obtained by using ciprofloxacin as immobilized template in a proof-of-the-concept study. The effects on the binding properties of the resulting nanoMIPs were studied by batch rebinding experiments, considering different experimental conditions in the SPPS protocol (different template scaffolding, pre-polymerization mixtures, and polymerization conditions) and different rebinding conditions (buffer composition, presence of organic solvents).

From the experimental results we found that the SPPS approach is highly flexible, and that resulting nanoMIPs show very good rebinding properties and excellent target selectivity in aqueous buffers, with equilibrium dissociation constants in the micromolar range. The extension of this approach to different analytical targets (mycotoxins, peptides, proteins) and the application of these nanoMIPs to the development of MIP-based immunoassays will be briefly discussed.

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O1 SB1

ATP SENSING PAPER WITH SMARTPHONE BIOLUMINESCENCE-BASED DETECTION

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ATP-driven bioluminescence relying on the D-luciferin-luciferase reaction is widely employed for several biosensing applications where bacterial ATP detection allows to verify microbial contamination for hygiene monitoring in hospitals, food processing and in general for cell viability studies. Rapid ATP kit assays are commercially available but the development of an ATP biosensor characterized by low-cost, portability, and adequate sensitivity where the reagents are immobilized is highly valuable to facilitate the early detection and rapid screening.
Thanks to low-cost wax printing technology and an innovative freeze-drying procedure, we developed a user-friendly, ready-to-use and stable ATP sensing paper biosensor that can be integrated in a portable light detector, including smartphone-integrated photocameras. The developed ATP sensing paper is able to quantify in 10 minutes as low as 10^{-14} moles of ATP in liquid samples. As proof-of concept, we analysed urinary microbial ATP as a biomarker of Urinary Tract Infection (UTI), confirming the capability of the ATP sensing paper to detect the threshold for positivity corresponding to 10^5 colony-forming units (CFU) of bacteria per mL of urine.

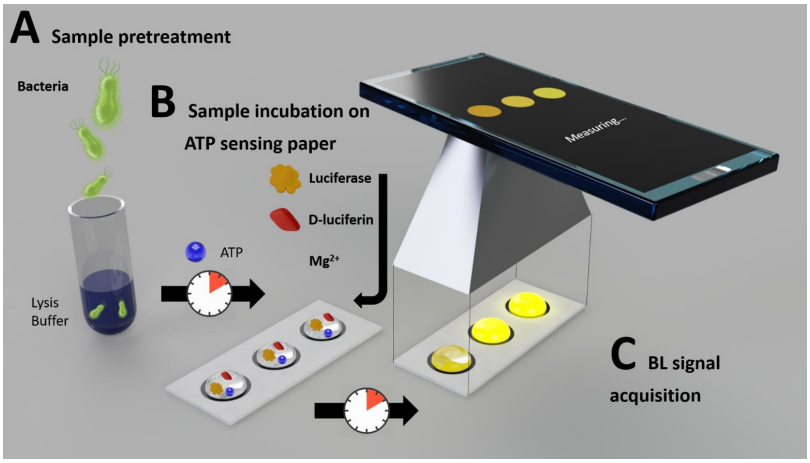

Figure 1. Schematic representation of the optimized ATP sensing paper assay.

O2 SB1

PNA-FUNCTIONALIZED MAGNETIC MICROBEADS AS SUBSTRATES FOR ENZYME-LABELLED AMPEROMETRIC GENOASSAY FOR GENETICALLY MODIFIED ORGANISM DNA SENSING

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Nucleic acid-based biosensors (genosensors) have received great attention in the last decade, being nucleic acids promising molecular probes due to the specificity for base pairing.
In a research program dealing with the development of innovative sensors as analytical tools for food safety assessment [1,2], we combined the performance of Peptide Nucleic Acid (PNA) probes with the efficiency of carboxyl-functionalized magnetic microbeads (mMBs) for the development of high performance genoassays with amperometric readout. The latter was carried out on glassy carbon screen printed electrodes (GC-SPEs) and on analogous electrodes based on embedded single-walled carbon nanotubes (SWCNT-SPEs).
The developed genoassays were applied to the determination of non-amplified DNA from genetically modified (GM) soy at trace levels.
A PNA-based Capture Probe (CP), with sequence complimentary to a 20-mer portion of "Roundup Ready" transgenic Soy DNA, was covalently immobilized on the active surface of mMBs, exploiting the reactivity of the carboxylic functionalities.
A signalling PNA probe (SP), complementary to a different portion of the target DNA and bearing a biotin tag, was used in combination with a streptavidin-alkaline phosphatase conjugate (ALP-Strp) to generate a three-probe sandwich leading to signals increasing with the target DNA concentration.

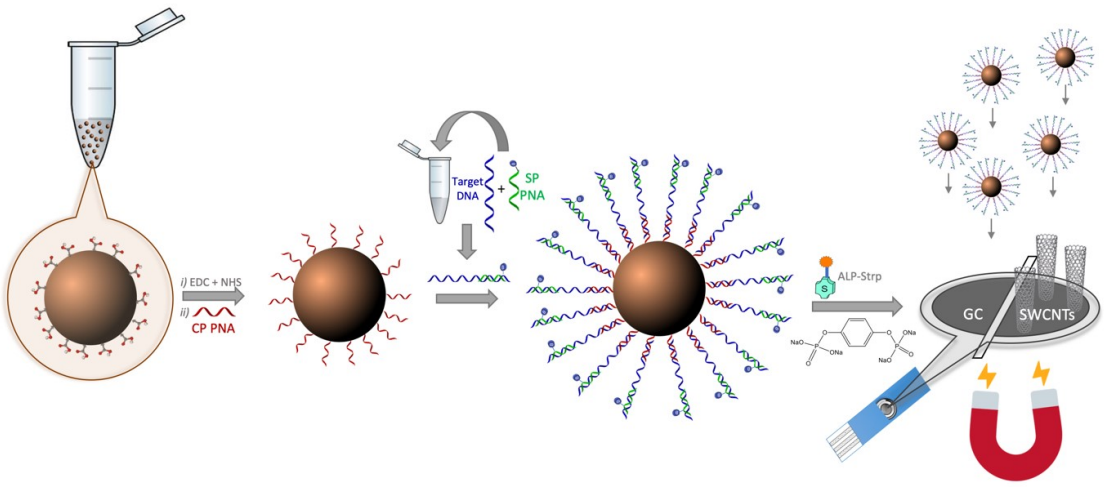

Figure 1. Working principle of the mMBs genoassay

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The performance of the developed genosensors was investigated by comparing the readout on GC-SPEs and SWCNT-SPEs. The intrinsic properties of the mMBs, which were exploited in terms of high reactive surface for the immobilization and hybridization of PNA probes, ensured in both cases an enhancement of sensitivity with respect to the performance exhibited by the corresponding newly developed and validated amperometric genosensors based on covalent immobilization of the same PNA probes on GC-SPEs or SWCNT-SPEs [3].
The best performance was achieved using SWCNT-SPEs for the readout, reaching limits of detection in the femtomolar range.
Finally, the methods based on the use of PNA-functionalized mMBs was validated on genomic DNA extracts from soy flour containing variable percentages of GM Soy, proving the discrimination capability of the genosensor based on SWCNT-SPEs towards GM material at trace levels.

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O3 SB1

ELECTROSPRAY IONISATION TECHNIQUE IN BIOSENSOR DESIGN: LACCASE AS CASE STUDY

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Immobilization of enzymes on the surface of transduction systems represents a compulsory and critical phase in the development of biosensors. Indeed, by influencing orientation, loading, and mobility of enzymes by means of tailored immobilisation procedures, it is possible to tune their structure and biological activity for achieving enhanced analytical performances in terms of sensitivity, selectivity, and stability. In the specific case of the laccase enzyme, this concern was elegantly dealt by Rodríguez-Delgado and co-workers in their review asserting that "*to become viable industrial catalysts, laccases need to be subject to treatments in order to make them robust, recyclable, or heterogeneous. One of the most studied treatments is immobilization, defined as attachment of an enzyme to an insoluble support. The benefits of an efficient protocol of immobilization are very important, namely prolonged use of the sensor and anticipated extended storage and working stability*" [1].
Herein, we described for the first time the use of electrospray ionisation (ESI) for the deposition of laccase on carbon black modified screen-printed electrodes (CB-SPEs) to design an amperometric biosensor for catechol detection. The ESI technique allows to bring large organic molecules as intact and isolated units in the gas phase, using a low-concentration solution of the molecule of interest flowed in a small capillary held at high voltage (4.5 kV) with respect to a grounded counter electrode placed 10 mm away. The charges on the liquid surface at the end of the capillary repel each other and expand at the solution/gas interface into a Taylor cone, provided the electrostatic force is counter-balanced by the surface tension of the liquid. When the surface tension cannot stand anymore the charges, a Coulomb explosion creates a spray of charged droplets whose size decreases as the solvent evaporates to form a gas of molecular ions. In these conditions the deposition of the molecule can be carried out at ambient pressure [2] and automatized, with significant

reduction of costs and times of the process in comparison with vacuum depositions [3]. In particular, the recent developments in the theory of electrospray process highlighted that there is a significant contribution coming from dielectrophoretic forces in addition to the electrophoretic ones, as a result of the inhomogeneous electric field (non-zero electric field gradient) typical of the experimental setup (where needle/target geometry is adopted). This would allow the process to take place even in the absence of neat charges rendering making it less stressing towards bio-molecules.

The aim of this work is to develop a standardised and reliable immobilisation protocol that preserves normal activity of the laccase enzyme, improving its working/storage stability and peculiar features for a biosensor by a commercial point of view. Laccase enzyme was deposed by ESI technique on carbon black modified screen-printed electrodes for the development of an amperometric biosensor for catechol detection. The choice of using carbon black as nanomaterial for SPE modification relied on the increased current signal of laccase towards catechol detection, which was 3 times higher in comparison to graphite SPEs, indicating the ability of CB to provide a larger surface area for laccase deposition as well as an increased conductivity that resulted in higher signals and thus enhanced sensitivity [4,5]. The performance of the laccase CB-SPE biosensor was tested via amperometric measurements at an applied potential of 0.160 V, in the presence of increasing amounts of catechol in a concentration range from 2.5 to 150 μM. Catechol was detected at a detection limit equal to 1.35 μM within a linear range from 2.5 to 75 μM described by the equation: $y = (0.04 \pm 0.02) + (0.102 \pm 0.005)\ x$ ($R^2 = 0.956$). The working stability of the biosensor was evaluated by testing the biosensor towards the detection of catechol at a concentration of 50 μM after repeated washes of the electrode, indicating a 100 % enzyme activity up to 27 measurements. The storage stability was appraised at room temperature in dry conditions, highlighting that the enzyme retained 100 % of the activity towards 50 μM catechol within a period of 180 days. Further biosensor analytical features are under investigation including interferent studies, matrix effect and recovery studies, to underline the potential of the proposed biosensor for real agro-environmental applications.

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O4 SB1

NANOMATERIAL-BASED SENSORS AND FOOD POLYPHENOLS: AN ANALYTICAL CHALLENGE AND A SOURCE OF USEFUL ELECTROCHEMICAL COMPOUNDS

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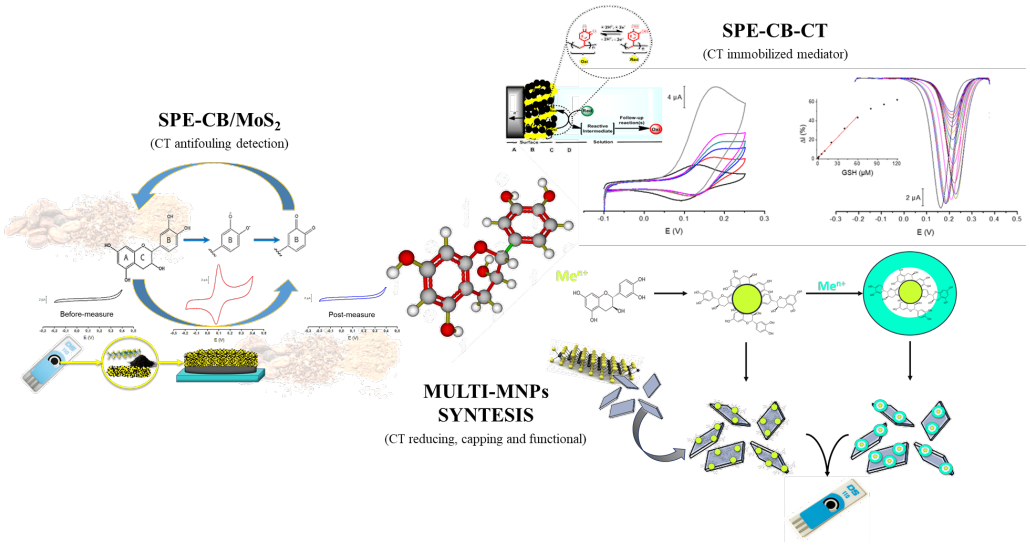
Nanomaterials (NMs) have become elective analytical tools able to improve the performances and give rise to new opportunities, for both constructions of analytical devices and implementation of analytical methods. On the other hands, polyphenolic compounds (PCs) still continue to attract exceptional attention, for their well-known health benefits, for their technological role and also marketing [1].
Thus, this work born with the idea to realize a regenerable and effective portable electrode able to directly allow the PCs quantification in foods. To this aim, different nanocomposites have been challenged, in particular, for the first time, 2D transition metal dichalcogenides (TMD) have been employed combined with carbon black nanoparticles, for the PCs analysis. In brief, a regenerable and totally anti-fouling (classical catechins/polyphenols analysis drawback) screen-printed electrode (SPE) based on TMD for the cocoa (CO) catechins (CT) rapid quantification has been realized, and at the same time, a new natural polyphenolic electrochemical mediator (CT) has been discovered, studied and exploited for a further nano-sensors realization.
Thus, firstly, the ability to detect catechins on a carbon black/molybdenum disulfide nanohybrid screen-printed electrode (SPE-CB/MoS_2) has been demonstrated. The SPE-CB/MoS_2 merge the ability of CB to improve the electrochemical response with the proprieties of MoS_2 to totally prevent catechins irreversible polymerization and absorption onto the electrode surface occurring at both bare and CB-modified SPEs. The latter, MoS_2 property has been proved in this study for the first time. The MoS_2 anti-fouling ability has been demonstrated using both flavanols standards and real samples. Moreover, the SPE-CB/MoS_2 proposed sensor allowed an improvement of sensitivity (LOD ≤ 0.17 µmol L^{-1}) of 100-folds compared to the bare SPE electrode, showing linear range between 0.12 and 25 µmol L^{-1} with good determination coefficients (R2 ≥ 0.998). 59 cocoa powder samples have been tested with the nanohybrid sensor developed and compared with the classical methods for polyphenols evaluation. The SPE-CB/MoS_2 allow to obtained repeatable (intra-electrode RSD = ip,a 0.9 % and Ep,a 5,2 %, n = 59) and reproducible (inter-electrode calibration slope RSD ≤ 4.1, n = 3) results, significantly correlated with classical methods for the polyphenols evaluation (r= 0.95–0.97). Noteworthy, after the measurements of 59 cocoa samples the electrode was still active (recovery signal 99 %).

O4 SB1

In a parallel track, during the study of nanomaterial reactivity towards cocoa catechins, carbon black demonstrated to act as catechins (CT) immobilization elective support. Indeed, upon the oxidation of CT to quinone, the cyclic voltammetry results demonstrated a pair of well-defined and reversible redox peaks. Surprisingly, the SPE-CB electrode after the CT measure (and after the washing-step), continues to show a pair of stable reversible redox peaks (RSD = ip,a 1.1 % and Ep,a 1,9 %, n = 20). Thus, was proven that the CT remain strongly attached to the electrode surface (SPE-CB) retaining a mediator-like electrochemical behavior. Thus, for the first time has been realized an electrochemical redox platform based on cocoa polyphenols, catechin-quinone (oxidized catechin), prepared by an in situ simple electrodeposition method onto a carbon black modified screen-printed electrode surface (SPE-CB/CT). The mediator (CT and cocoa extract) activity, has been studied, proved, and applied to glutathione (GSH) detection. The optimized SPE-CB/CT sensor exhibited a linear response to glutathione concentrations from 5 nM to 100 µM. As proof of concept, the fabricated SPE-CB/CT sensors, realized directly using cocoa extracts, were successfully employed for the determination of GSH and oxide glutathione (GSSG) in biological fluids.
In order to further prove the exploitability of the interaction between NMs and PCs, works are still in progress for the realization of TMD-based electrochemical platforms decorated with bimetallic nanoparticles, synthesized exploiting the PCs unique features. Definitely, this work contributes to further prove that nanomaterials (in this case TMDs and CB) are unique and useful analytical tools able to both tailor 'customize sensors' and giving rise to new analytical opportunities.



Graphical scheme of the main topics treated in this work

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O5 SB1

DNA-BASED RECEPTORS CONTROLLED BY REDOX INPUTS

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Synthetic DNA is an extremely versatile and programmable building block often employed to engineer nanoscale supramolecular devices with potential applications in the field of diagnostic and drug-delivery. Despite the wide range of inputs used to control DNA-based reactions and nanomachines (pH, small molecules, enzymes, antibodies or metal ions) the possible use of redox inputs has seen very limited efforts. This appears surprising considering the importance of redox regulation inside the cell. Redox regulation is in fact one of the most important and plays an essential role, not only in cell survival, but also in cellular signalling systems. Motivated by the above arguments, we propose here a new strategy to rationally modulate the activity of DNA-based nanodevices through redox inputs. We demonstrate the possibility to control the loading and release of a molecular cargo using redox inputs in a reversible and transient way and we also demonstrate the capability to modulate this kind of control. The possibility to use redox inputs will expand the available toolbox of molecular stimuli and could open the door to many applications including redox-triggered nanostructures assembly.

O1 CH

BPA-FREE EPOXY RESINS FOR STONE CONSERVATION: SYNTHESIS AND NEW ANALYTICAL APPROACH TO OPTIMIZE THEIR CURING

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The development of stone conservation products is an active field of research because the achievement of environment-friendly multifunctional products able to ensure, at the same time, long-term key properties such as consolidating and hydrophobic effects, is a hard task. In this sense, novel products derived from Bisphenol A (BPA)-free epoxy resins have become increasingly appealing due to the easy tailoring of their physical, thermal and chemical properties coming from their combination with compatible inorganic precursors and/or their nano-reinforcement [1].
The aim of this work was double: on one side the synthesis of a BPA-free epoxy resin, based on a diglycidyl ether of a substituted cycloaliphatic diol, with lower health and environmental concerns with respect the widespread phenolic-based epoxies; on the other hand, the development of a new spectroscopic methodology to follow and optimize the synthesis and curing processes.
Accordingly, the synthesis of 2,2,4,4-tetramethyl-1,3-cyclobutanediol diglycidyl ether (CBDO-DGE) was carried out from the reaction of 2,2,4,4-tetramethyl-1,3-cyclobutanediol (CBDO), 2-(chloromethyl)oxirane (EHC) and sodium hydroxide (NaOH) in the presence of a phase transfer catalyst, tetra-n-butylammonium bromide (TBAB) (Figure 1) [2].

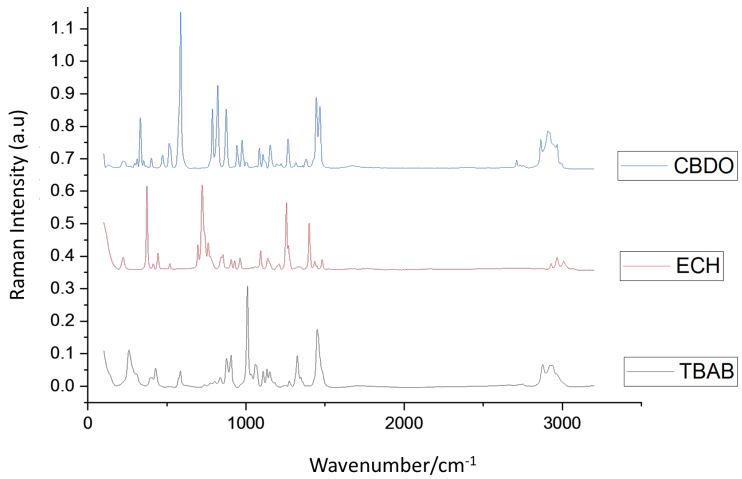

Figure 1. Raman spectra of reactants employed for the epoxy synthesis.

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The crude purification method was followed by FT-IR and Raman spectroscopies, whereas the characterization of the expected compound was carried out by ^{1}H-NMR and ^{13}C-NMR investigations. Then, the ratio epoxy-amine used for its thermal cure was optimized with the help of attenuated total reflection (ATR) measurements and chemometric treatment of the obtained data with The Unscrambler software, using multivariate analysis methods such as Partial Least Squares (PLS) or Multivariate Curve Resolution (MCR) [3]. The thermal behavior of the most promising product, to be exploited as stone conservation material, was studied by means thermogravimetric (TGA) and differential scanning calorimetry (DSC) investigations. Preliminary results coming from the application of such product on selected stones are also discussed.

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O2 CH

ISOTOPE RATIO MASS SPECTROMETRY: OLD AND NEW PROXIES IN CULTURAL HERITAGE RESEARCH

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Isotope ratio mass spectrometry (IRMS) is an analytical technique whose practice continuously spreads in disciplines such as medicine, geology, biochemistry, food authenticity, ecology and forensic science. Alongside, cultural heritage researches benefited from the introduction of these isotopic proxies which increased the awareness on environments and human activities in the ancient times. Climatic and environmental reconstruction, human diet and movement, crop management practices, pottery use are some of the new trend topic in archaeological research that have been advanced thank to IRMS.
Both compound specific isotopic analysis (GC-C-IRMS) and bulk isotopic analysis (EA-IRMS) are conducted in our Laboratory. The former, for example, helped to reconstruct the diet [1,2] or rituals [3,4] in different archeological sites.
Essential to understand past diet and mobility is to know how isotopic signals are transferred from the environment through the trophic levels. EA-IRMS model studies on selected cultivation permitted to assess if archaeological findings were wild or watered crops [5] which helps in elucidating the onset of agriculture, a key moment in the human past.
Petroleum products such as bitumen or asphalt were exploited by ancient populations for several uses such as to repair broken pottery up to build the huge Birs Nimrud ziggurat. Together with the more archaeologically significant bitumen deposits in the Near and Middle East [6], there are several seepages in Central Mediterranean which deserve more attention with respect to their utilization and circulation in Antiquity. Biomarker based approaches were successfully used to provenance bitumens both directly [7] and after principal component analysis [8]. EA-IRMS has also been used along with biomarkers to succeed this task [7,9]. However, a misuse of stable isotope is possible. Our work on Neolithic and Bronze Age bitumens, indeed, showed that the isotopic signature on selected fractions cannot be uncritically used as parameters to genetically correlate source rocks and provenance bitumens. On the contrary they represent a new proxy in cultural heritage research highlighting use or processing [10].

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O3 CH

AN ANALYTICAL BASELINE FOR RED-COLOURED TEXTILES OF PHARAONIC EGYPT

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The image of an Egyptian mummy is normally one of a human body wrapped with pallid-coloured linen bandages. However, in spite of this image which endures in the public imagination, some mummies in the first millennium B.C. were covered by red shrouds or wrapped with red-coloured bandages. Some of these shrouds have fully retained their intense colour over time, and in some other cases red-coloured textiles become visible by removing external straps or other mummy bindings to expose the under-laying textiles. This is frequent during conservation treatments, which allow us to take a look at the colourful reality hidden under textiles that - apparently – have the colour of natural flax fibres.
Although some general information on likely candidates for colour are reported in the archaeological literature [1], a systematic scientific study, aimed at defining the general strategy for the detection of the colouring materials in ancient Pharaonic textiles, has so far been lacking. A set of analytical approaches have been explored in this work in order to define the most suitable tools to support archaeologists in shedding light on how the use of red shrouds and textiles was embedded in the funerary practices of Pharaonic Egypt.
The collaboration between analytical chemists and archaeologists yielded a set of 15 samples, which were obtained from mummy bandages and shrouds from the collections at the Museo Egizio in Torino and at the British Museum in London. The scientific team investigated the colourants *in situ* by non-invasive techniques (namely, fibre optic reflectance spectroscopy - FORS - and portable fluorimetry, p-FL), and in the laboratory according to a micro-invasive approach. Optical microscopy (OM) under visible or UV illumination, HPLC-ESI-Q-ToF, SEM-EDX and micro-XRF were employed on the samples detached from the selected textiles. The analytical approach was primarily aimed at highlighting the advantages and drawbacks of each technique regarding the identification of

colourants and dyeing methods. The investigation of inorganic mordants was considered throughout, particularly because these textiles are normally heavily contaminated and data from elemental analyses are open to misinterpretation. Overall, the main aim of the study was to establish an analytical baseline over which further case studies may be assessed and added to in the future.

Figure 1. The red shroud on mummy S. 5227 (Torino, Museo Egizio).

The discolouration of some of these textiles represents a further intriguing aspect, as in some cases the red colour is still vivid and well-preserved (Figure 1), whereas in others it appears faded or severely discoloured.
Results pointed towards the presence of at least three sources of the red colour: safflower (*Carthamus tinctorus*), madder (*Rubia* sp.) and a red ochre. As expected, the information obtained by HPLC-MS enabled more detailed information to be collected when organic dyes are present, with high sensitivity and detailed identification of mixtures of dyes. On the other hand, both FORS and UV-OM microscopy proved to be very effective and affordable tools for an informative screening of the colouring materials. FORS can provide conclusive identification of the main colouring agents, and UV-OM is able to distinguish between luminescing (madder and safflower) and absorbing (red ochre) materials.
The investigation of the inorganic components by comprehensive analysis on the whole fibre by micro-XRF and spot analysis on single particles by SEM-EDS enabled some information on mordanting to be recovered, although the unequivocal recognition of the raw materials possibly used for fixing the dyes proved to be a hard task.
The combination of micro-morphological observations and elemental analyses yielded some insights into the colouring processes and allowed us to highlight the most fugitive colour. Textiles dyed with red ochre or madder still preserve, at least partially, their red colour. For madder, the scientific insight lead to conclude that light fastness was improved by exploiting the combined effect of alum and tannins in a same dye-bath to produce a sort of “madder lake” deposited on the fibres. As expected, poor light-fastness of safflower resulted in the almost complete fading of the areas that have been exposed to light.

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O4 CH

ANCIENT BILE ACIDS IDENTIFIED BY HPLC-ES-MS/MS IN SOIL/FAECAL SAMPLES, COLLECTED IN A NEW ARCHEOLOGICAL SITE OF SUSPECTED ROMAN SEWER AND LATRINES IN POMPEII RUINS

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Among the different markers used in archaeology and related disciplines for detection and source identification of faecal matter in soil [1], steroid analysis is a promising tool when the archaeological context regards the potential presence of faecal sample, under oxygen-deficient conditions like in latrines or sewer system. Steroids are stable molecules well preserved and therefore used as biomarkers for a faecal input that occurred even in ancient era of thousands of years ago [2,3]. Particularly neutral steroids such as stanols and stanones (Δ5-sterols) can be used for the presence of faeces and faecal inputs into soils and sediments [4]. On the other hand, the ubiquitous occurrence of Δ5-sterols (dead plant or animal material, root exudates, faeces, or soil micro- flora and fauna), as well as their transformation to stanols in the environment, reduce their specificity as mammalian faecal biomarkers. The commonly used steroids still do not take advantage of the full potential of the steroid spectrum, because they do not consider bile acids (BAs). BAs are likely the most specific steroids markers for a faecal input, due to their exclusive occurrence in vertebrate faeces. Furthermore, BAs are more resistant to degradation than Δ5-sterols, stanols, and stanones [5] and can therefore still reveal an ancient input into soils where other markers have already been degraded or further metabolized. BAs are acidic steroids synthetized in the liver from cholesterol, are excreted into the intestine and then transformed to secondary BAs by intestinal microflora. In the human body most of the secondary BAs like lithocholic acid (LCA) and deoxycholic acid (DCA) are excreted in faeces. Due to different BA synthetic pathways and metabolism, BA profiles of vertebrates (including humans) may differ significantly [6].

Secondary BAs undergo further several metabolic pathways by gut microbiota to produce epimers and oxidized derivatives. Oxo bile acids (oxo-BAs) are present in faeces at levels similar to those of their metabolic precursor, up to 20-30 % of the total faecal BA [7]. Among mammals, faecal BA composition is therefore specie-specific and their qualitative profile can be used as powerful specific tool to identify the source of faecal samples and verify possible cross-contamination. We have developed and optimized an HPLC-ES-MS/MS method for the analysis of up to 21 BA and their oxo-BAs metabolic products in faeces. Efficient solid phase extraction and concentration procedure have been developed for BA extraction from soil

with high recovery, in order to obtain detectably concentration when the original faeces are dispersed and diluted in the soil sample of sewer and latrines. The present method was accurate (bias%<15%), precise (CV%<10%) and sensitive (LOD<30ng/ml). The MRM detection mode provided high selectivity and minimized matrix effect

This method was applied to Pompeii's soil samples from a latrine pit of a toilet, collected during recent archeological excavations in Obellio Firmo's House, to assess the presence of fecal matter.

The main BA identified in the samples were deoxycholic acid and lithocholic acid with a mean concentration of 54 ng/g and 12 ng/g respectively. The BA profile and the ratio between BA and Oxo-BA levels (about 3:1) has been used to assess the human origin of the feacal input in the archeologic samples.

The results from the analyzed samples confirm the discovery of a toilet site in Pompeii ruins, assessing the human origin of the fecal material. To our knowledge, it is the first time that Oxo-BA metabolites have been identified and measured in ancient samples.

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O5 CH

A NEW MINIATURIZED NEAR INFRARED SPECTROMETER FOR ON-SITE CULTURAL HERITAGE INVESTIGATIONS

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In the last decades, the technology of near infrared (NIR) spectrometers has grown rapidly by moving from laboratory benchtop instruments to portable devices that can be used on site. Subsequently, NIR spectroscopy has acquired increasing interest as a diagnostic tool for the non-invasive study of works of art in art galleries or museums, thus avoiding the movement of precious and fragile objects. However, the scenario of applications of NIR spectroscopy for the study of heritage objects are still wide and poorly explored. One of the most fascinating perspectives is the development of new compact systems for easy use on site also by conservators and restores. The miniaturization and cost reduction of spectrometers responds to end-user and producers' inquiries and are showing great potential to open new research topics in analytical technologies.
In the present research a new miniaturized NIR spectrometer prototype was proposed and evaluated for the characterization of different cultural heritage materials, such as paintings, bronze patinas, archaeological bones and cinematographic films. The NIR prototype system enables rapid information acquisition to guide restoration strategies, which must be supported in real time by quick and easy analytical procedures. Appropriate reflectance spectral databases, as well as tailor-made chemometric methods for classification and identification of materials, were implemented.
Among others, results show that the system, together with a multivariate analysis of spectroscopic data, is a reliable and fast method for the analytical characterization of historical film bases. The cinematographic films are constituted in general terms by a transparent polymeric base (consisting of cellulose nitrate, cellulose acetate or polyethylene terephthalate), whose correct identification is of fundamental importance to select the appropriate storage conditions.
The spectrometer has also been shown to be effective for the preliminary identification of collagen in bones. Indeed, rapid and non-invasive screening methods are usually needed to detect collagen in bones before being subjected to proteomic, radiocarbon and isotopic analyses to obtained information on identity, period, and diet/environment of ancient population and animal species.

O1 EAC1

EMERGING ORGANIC CONTAMINANTS IN INTERIORS

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Recently, emerging contaminants (ECs) have gained the concern of environmental scientists. This class of toxicants is overall typical of indoor environments; aside of work places, interiors of dwellings, public buildings, offices and shopping centers are involved; there, people spend most of the life time (80-90% of the total). In interiors, ECs reach concentrations much higher than outdoors, giving raise to exposure rates up to thousands times higher. Even the way of toxicants' intake changes; indeed, while at open air inhalation is predominant, indoors both ingestion and contact gain importance.
ECs display a wide variety of chemical properties and toxicity forms. Most EDs are organic displaying semi-volatile properties, occur in a number of healthcare and house products, in foods, furniture and building materials; usually EDs are not carcinogenic but promote allergies, immune depression, irritation and other acute effects, endocrine system damages, as well as chronic diseases like diabetes, infertility and psychologic problems. Among ECs, key roles are played by plasticizers (e.g., phthalates and adipates), flame retardants (PBDEs, organic phosphates),. Worth of note, EC chemicals occurring in ambient air and surfaces are distinct from those affecting waters (fluorinated surfactants, alkyl ethoxylates, drug by-products).
No attention is usually paid to psychotropic compounds (with illicit drugs) as well as to pharmaceuticals, analogously to cosmetics whose formulas include a list of anti-oxidants/UV light shields and additives (parabens, alkylphenols, bisphenols; fragrances and siloxanes) ascertained as harmful. Interestingly, these categories affect air in the native form, unlike waters, sewages and wastes, where they occur overall as degradation products [1]. Almost no investigations have been conducted till now and cumulative data bases are poor.
To improve the knowledge at this regards, an extensive study has been undertaken in Italy aimed at identifying a list of target substances (crossing toxic properties and environmental occurrence of ECs, with special focus on drugs and pharmaceuticals), at optimizing the sampling and chemical analysis procedures for both gaseous and particulate chemicals based on bench-top GC-MSD techniques), and at acquiring information about their loads and behaviours in interiors, through in-field measurements conducted at schools, dwellings, offices, labs and hospitals.

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O2 EAC1

SELF-ORGANIZING MAP AS A SUPPORT FOR DATA FUSION IN MONITORING OF ENVIRONMENTAL PROCESSES

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Environmental processes are often characterized by dynamics that can be described by multiple heterogeneous measurands or indicators. Complexity of phenomena can be handled only considering multidimensional information, that is collected by different criteria (e.g. frequency, instruments), whose content need to be visualized synoptically.
Multi-sensor (and multichannel) technologies allow to follow variability of environmental phenomena with relatively high frequency (e.g. minute), as it is the case of odorous gas immissions monitored by Instrumental Odour Monitoring Systems [1] and of air particulate matter measured by Optical Particle Counters [2]. In environmental monitoring close to industrial sources, productive cycles as well as temporal periodicity (e.g. night/day cycles, working days/week ends, seasons) imply that similar signal patterns from multi-sensor systems occur repeatedly. The training of artificial neural networks known as Self-Organizing Map (SOM) algorithm, allows to identify a finite number of recurrent signal patterns (aka neurons or prototype vectors) that are organized in a bi-dimensional lattice (map) accordingly to their similarity. Prototype vectors can be clustered, leading to identification of typical states of the dynamic system under study. Every new signal pattern from the environmental monitoring can be projected on the map, identifying the best matching unit (BMU). A correspondence between each monitoring time and a neurons of the map is thus established, which is foundation for the data fusion procedure. Data from external sources – e.g. physical and chemical air quality parameters measured by means of standardized methods, measured with their own frequency, but also sparse data as citizen complaints, and plant faults - are linked to the BMU of synoptically collected multi-sensors patterns, and thus to clusters and typical states of the system. The finite number of typical patterns and system states is characterized on the SOM - thanks to the data fusion procedure - as normal/background states or states of the system polluted in different degree or from different sources. The adequacy of the trained SOM to represent new data, can be checked by examination of their quantization error (QE). Diverging QE indicate opportunity to check the systems for eventual hardware faults and to re-train the map. Trajectories of the system from normal to polluted states and back can be followed, making the SOM a multivariate control chart suitable for handling data from complex environmental systems. Till now the monitoring of odour [1] and PM [2] issues have been considered, but the approach appear suitable to analyze and visualize effectively multivariate data from long term monitoring of general environmental and industrial processes.

O2 EAC1

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O3 EAC1

THE DEGRADATION OF REAL SAMPLES OF PLASTIC BOTTLES FROM ADRIATIC MARINE LITTER: VIBRATIONAL SPECTROSCOPY EVALUATION

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In the framework of the compelling problem of anthropogenic debris negatively affecting the marine ecosystems, with a strong impact on marine water quality and life, many studies were carried out in the last decades concerning plastic degradation with the aim of understanding the processes occurring to plastic litter in the marine and coastal environment. The investigation on degradation can be directed toward the comparison of compostable plastics with respect to conventional ones, to the evaluation of plastics recyclability, or to an estimation of the potential generation of secondary microplastics [1, 2].

Notwithstanding, up to now, most of literature studies concerned degradation of standard polymers references as induced by artificial weathering conditions, mainly through exposition to UV radiation, heat or/and microorganisms (photo-, thermo-, bio-degradation). In some cases, natural outdoor ageing and/or immersion in saline water were also experimented [3], mostly using standard polymers or, more rarely, non-degraded objects. Very few works were carried out on real marine litter (ML), one of which performing ATR-FTIR measurements on polyethylene terephthalate (PET) bottles, taking advantage of the temporal sequence provided by the expiration dates still present on some sampled bottles [4].

Within the Italy-Croatia Interreg "ML-Repair" (REducing and Preventing, an integrated Approach to Marine Litter Management in the Adriatic Sea) project, a selection of about 150 marine litter bottles was examined. These were a result of Fishing for Litter (FfL, the removal and correct disposal of ML recovered during fishing activities) practiced from Italian Northern Adriatic and Dalmatian (Croatia) channel waters and open waters. A systematic investigation was carried out on PET bottles bodies and necks – parts that undergo different manufacturing and are also differently exposed to the environment - and, where present, on their caps and seals, made of HD-PE (high density polyethylene). This allowed a parallel study of two polymers evolution in the marine environment.

After preliminary phases of cleaning, decalcification and removal of biogenic incrustations, Attenuated Total Reflectance Fourier Transform Infrared (FTIR-ATR) and Raman (excitation wavelength: 785 nm) Spectroscopy were performed acquiring three spots of analysis for both the interior and the exterior sides of each of the four parts of the bottles. The obtained spectra were compared with those of references of virgin PET and HD-PE, and with those acquired on similar new bottles, caps and seals of different colors. This was done in order to

take into account also additives and colorants and their influence on the acquired spectra. Furthermore, the results obtained showed that most of the new bottles display as well on their external surface some signals generally attributed to effects of photo- or thermo-oxidation, as reported in Figure 1 for PE ATR-FTIR spectra, where 1712, 1740 and 3300 cm^{-1} signals are highlighted [5]. This information is important to evaluate the real extent and the type of degradation occurring on plastic bottles in the marine environment. The spectroscopic data collected by FTIR-ATR on ML bottles also show a marked biodegradation [6] (Fig.1c) and indicate that the bottle necks interiors are the least degraded sections.

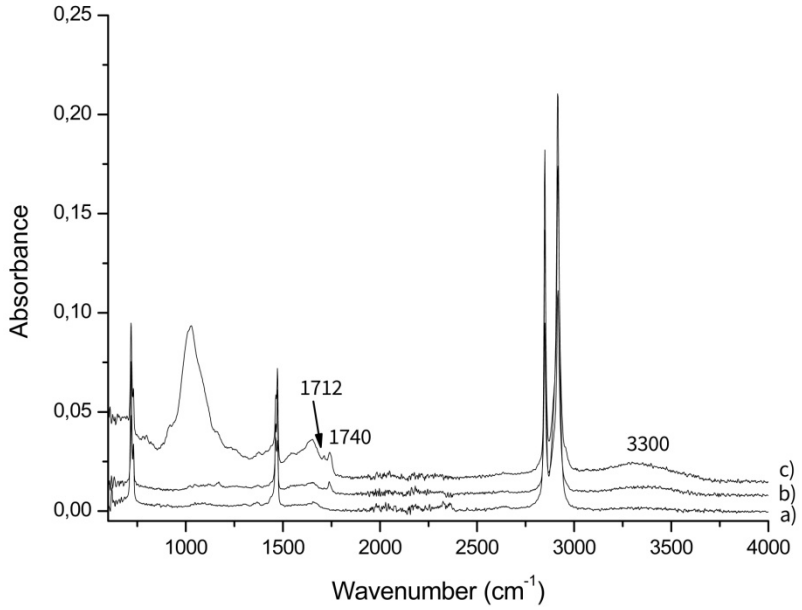

Figure 1. FTIR-ATR comparative spectra of virgin HD-PE (a) and of the external surfaces of a white cap of a new bottle (b) and of a ML one (c), respectively; an offset was applied for an easier visualization.

The Raman spectra of ML samples, though interestingly different according to the colors and the parts analyzed (body or neck), resulted strongly affected by fluorescence, especially where the biodegradation is more intense.

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O4 EAC1

USE OF NANO-STRUCTURAL MATERIALS FOR POLLUTANTS REMOVAL IN WATER TREATMENT

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Intensive cultivation and industrial activities inevitably leads to water and soil pollution. Intensive agricultural production requires the use of specific fertilizers with superior and technical characteristics to conventional ones. Among them, the most efficient and functional fertilizers are slow-release ones. A significant issue of the Italian territory is the leaching of nutrients brought into the soil, such as nitrates and other anions. This phenomenon does not allow nutrients to be assimilated by plants, but end up directly in groundwater or in natural waters. Concerning the industrial activities, groundwater pollution can be accidental or malicious. Main pollutants that could be found into groundwaters are organic compounds (such as pesticides) or heavy metals. To try to solve these big issues, the study was focused on innovative nano-structured materials (such as nanosponges) for the removal of different types of pollutants. Nanosponges result from maltodextrin based biopolymers. Maltodextrins are a family of oligosaccharides achieved from starch coming from different biomass (such as potatoes, corn, peas). The use of specific cross-linking agents allows to get a hyper-cross-linked biopolymer having an eco-compatible and biocompatible polymeric structure. Moreover, it is possible to have a high retention and release of relevant compounds, according to appropriate synthetic modifications. The syntheses have been optimized towards a greener process, using water as a reaction medium and without any use of organic solvents.

Laboratory tests were performed to assess the capacity and potential retention of heavy metals, organic compounds and anions (such as NO_3^-). In the specific case of nitrates removal, nanosponges were evaluated as slow-release fertilizer. Several absorption tests were conducted (both in batch and in column), changing concentration, absolute quantity and volume. The results achieved were from 50% up to 80-90% retention of anions (including nitrates) and heavy metals (including Cr (VI)). Concluding, nanosponges are innovative, biodegradable and eco-compatible sugar-based materials that have shown noticeable potential for future application in water treatment.

O1 BIO1

LONG-TERM, IN SITU SENSING OF MICRORNAS ENABLED BY NANOCOMPOSITE FIBER NETWORKS SHELTERING DNA MOLECULAR BEACONS

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In vivo sensing applications demand extended residency time and localized release of the functional probes for overcoming the constraints of multiple interventions. It is especially challenging to endow tissue engineering scaffolds with sensing properties, as these often require residence times ranging from several days to months in order to perform their function. This currently limits the extent to which DNA-based probes can be deployed in tissue engineering and *in situ* molecular detection [1]. This work presents a strategy for bypassing the above limitations using hierarchically assembled platforms in which a DNA molecular beacon is incorporated in mesoporous nanoparticle carriers embedded in a polymer nanofiber matrix [2]. We show that these multiscale scaffolds can be engineered to provide controlled and sustained release of a DNA probe for long-term detection of a target microRNA marker. First, model synthetic DNA is loaded into porous silicon nanoparticles (pSiNPs) using a calcium-silicate trapping method, then incorporated into polymer nanofibers by means of a spray nebulization technique. The resulting hybrid nanofibers are characterized for their ability to release the oligonucleotide payload under under temperature and pH conditions mimicking physiological values. The amount of DNA released scales with the quantity of DNA-loaded pSiNPs and the chemical nature of the fiber matrix, which allows for arbitrarily tuning the release between 5 and 20 days. Next, we demonstrated that a DNA molecular beacon designed to recognize microRNA-21 (miR-21) can be used as a sensing payload retaining its functionality during extended timeframes. *In situ* detection of the target microRNA can be achieved at programmed time-points with defined signal gains spanning 20 days. This works shows that microRNA sensing can be performed in situ and in real time by combining miRNA-responsive DNA molecular beacons with hybrid polymer/porous silicon fiber scaffolds, suggesting that extracellular microRNA markers may be detected directly in cell culture over several weeks of incubation.

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O2 BIO1

ORTHOGONAL REGULATION OF DNA NANOSTRUCTURE SELF- ASSEMBLY AND DISASSEMBLY USING ANTIBODIES

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Here we report a rational strategy to orthogonally control assembly and disassembly of DNA-based nanostructures using specific IgG antibodies as molecular inputs. [1] To do this, we first demonstrate that the binding of a specific antibody to a pair of antigen-conjugated split input-strands induces their co-localization and reconstitution into a functional unit that is able to initiate a toehold strand displacement reaction. [2] The effect is rapid and specific and this approach results versatile and in principle, generalizable to any antibody for which an antigen can be attached to a DNA anchoring strand. In support of this claim, we have demonstrated that two different DNA circuits can be controlled in an orthogonal way in the same solution by using two different IgG antibodies as Inputs. Such antibody-regulated DNA-based circuit has been then employed to control the self-assembly of two distinct DNA tubular structures by using DNA circuits controlled by two different IgG antibodies in the same solution in an orthogonal way. Similarly, we can autonomously control in the same solution [3] the assembly of a DNA nanostructure with a specific antibody and trigger the disassembly of the same structure with another antibody. Given the growing importance of antibodies as molecular markers in diagnostics and as therapeutic drugs, the antibody-controlled DNA nanostructures we report here may prove of utility in a range of applications, including controlled drug-release, point-of-care diagnostics and in-vivo imaging.

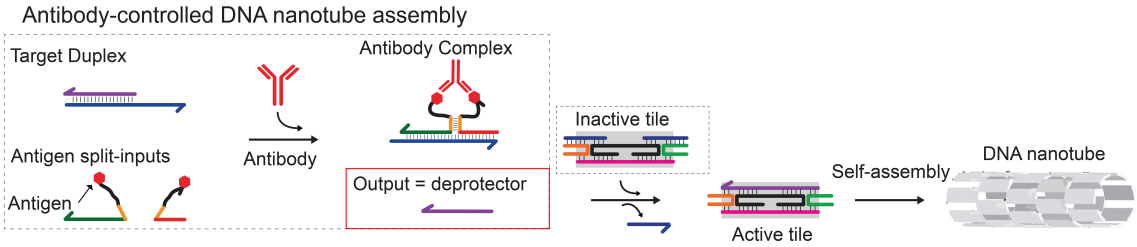

Figure 1. Antibody-controlled DNA circuit re-engineered to trigger the assembly of DNA nanotubes.

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O3 BIO1

DEVELOPMENT OF AN INNOVATIVE ANALYTICAL PLATFORM FOR ENRICHMENT AND IDENTIFICATION OF SHORT PEPTIDES IN BODY FLUIDS

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Body fluids are complex mixtures of metabolites of various chemical nature and thus significant sources of biomarkers for human diseases.[1] Despite being frequently described as potential biomarkers[2], short endogenous peptides still lack of proper strategies for their analysis, as they represent an analytical challenge due to their inhomogeneous chemical-physical properties, poor ionization efficiency using electrospray-mass spectrometry (MS) for their determination and the absence of automation in their detection. In order to overcome those issues, an innovative platform for enrichment, separation and identification has been developed. First, an enrichment strategy based on graphitized carbon black (GCB) was tested and developed on a mixture of analytical standard peptides which was representative of the naturally occurring peptides in body fluids. Short peptide enrichment was necessary due to the low abundance of those substances and the extreme complexity of body fluids samples. Ultra high performance liquid chromatography separation was carried out using two orthogonal chromatographic strategies, namely reversed phase (RP) C18 and Zic-HILIC columns, in order to maximize the number of identified peptides. A suspect screening approach was chosen for high resolution MS coupling.[3] In particular, a database of all the amino acid combinations for short peptides was compiled and MS/MS fragmentation was only performed on precursor ions matching with those in the database, resulting in a significant boost in sensitivity. Finally, MS/MS spectra were manually matched to spectra generated *in silico* to confirm the identity and the correct amino acid sequence.
The method was applied to the investigation of short endogenous peptides in human urine and plasma from healthy individuals resulting in the identification of 161 and 41 amino acid sequences, respectively. To the best of our knowledge, this is the first proposed method for enrichment and identification of short peptides in body fluids samples.

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O4 BIO1

HPLC-HRMS AS A TOOL FOR AUTISM SPECTRUM DISORDER BIOMARKER SEARCH IN BLOOD SAMPLES

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It is recognized that dyslipidemia plays a role in neurodevelopmental syndromes including the so called Autism spectrum disorder (ASD), a broad and heterogeneous group of neurological developmental disorders classified according to Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Since ASD involves mainly problems with social skills, communication and repetitive pattern of behaviors to date, it is diagnosed by psychologists because of the absence of reliable chemical biomarkers [1]. The aim of this preliminary work is to search for putative biomarkers in blood easily collected in non-invasive way from young ASD patients. After blood treatment on a ficol gradient, blood mononucleates (lymphocytes and monocytes predominantly) are separated from plasma. So, the composition of lipid species in plasma and lymphocytes has been examined by using hydrophilic interaction liquid chromatography (HILIC) coupled with electrospray ionization and Fourier-transform mass spectrometry (ESI-FTMS) [2]. The study has been carried out on samples obtained from kids (age ranged between 3 and 16 years) affected by ASD with severity degree from 1 to 3 without any pharmacological treatment and from their unaffected brothers or sisters considered as healthy subjects.

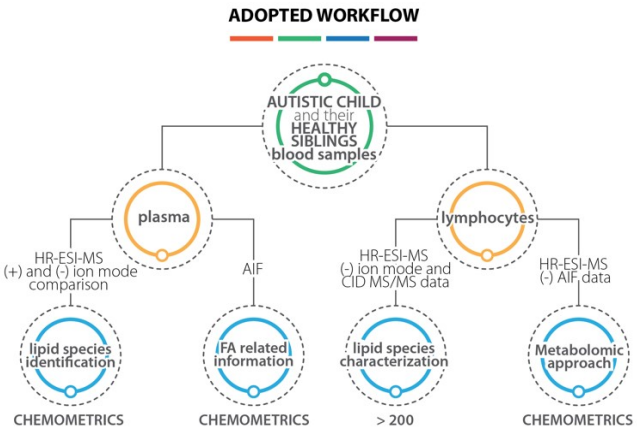

Figure 1. Adopted Workflow for lipid biomarker discovering in ASD patients

O4 BIO1

The LC separation by HILIC column allowed phospholipids separation according to their polar head; in this way, lipid species from different classes having the same nominal mass, often almost co-eluent due to their side chains structural similarity in reversed phase chromatographic (RPC) separations, are well separated and non-ambiguous identification are possible by using high resolution mass spectrometry [3]. To manage the large amount of data obtained using this untargeted lipidomic approach (i.e. the comprehensive analysis of all the measurable lipids in a sample and lipid abundance inferred from the arbitrary intensity values usually normalized to each class), Alex ^{123} [4] software was employed.
For plasma samples, comparison between positive and negative ion mode spectra, together with orthogonal information provided by lipid class retention time, allowed a confident assignment of extracted lipids. The application in positive ion mode of the orbital-trap exclusive fragmentation process, called “all ion fragmentation” (AIF) where all ions are fragmented without precursor ion isolation allowed lipid class confirmation, while in negative ion mode information on fatty acid composition can be retrieved; so, phospholipids and fatty acids levels were compared among siblings using paired t-test.
In the case of lymphocytes, the regiochemical characterization was accomplished on more than 200 lipid species, including phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols and their lyso-forms, sphingomyelins and glycolipids. Despite metabolomic approach is quite common in biomarker discovery [5], there are very few examples in literature of this approach using AIF data [6]. Upon implementing a proper database, Alex^{123} software was exploited and chemometric examinations were applied for biomarker discovery. Here we demonstrated the potentiality of this combined data mining in highlighting small differences hidden with classical metabolomic approach, but which would be fundamental for biomarker discovery in so complex and still enigmatic pathology.

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O1 FC

A PRELIMINARY STUDY TOWARDS THE DEVELOPMENT OF AN INNOVATIVE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY-BASED PROTEOMICS STRATEGY FOR SKIN WOUND AGE ESTIMATION IN FORENSIC MEDICINE

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In forensic pathology wound age estimation is a classic but still modern issue that meets the need of establishing a causal relationship between any wound and death. In fact, if in autoptical observation it is quite straightforward to localize and identify wounds, on the other hand the assessment of the time intervening between the injury and death is anything but trivial. In the last decades many efforts have been paid for the selection of wound healing-related molecular biomarkers as the most effective for wound age estimation [1, 2] and adequately supported by scientific evidence to be a proof value in court. Immunohistochemical analysis is the commonly exploited method applied to formalin-fixed paraffin-embedded tissue, offering the possibility to localize the biomarker within the tissue or cell substructures, giving only qualitative or semi-quantitative results. In addition, it suffers from operator-related manual variability, subjective data interpretation, artefact generation risk, low sensitivity, and difficulty to visualize target that are co-localized [3, 4].
For these reasons, forensic pathologists urgently require more reliable and sensitive analytical approaches for wound age estimation. In this context, to the best of our knowledge, the present investigation represents the first attempt to develop a high-throughput bottom-up proteomics strategy based on liquid chromatography/tandem mass spectrometry (LC-MS/MS) applied to autoptic skin for the development of a target method for the simultaneous determination of biomarkers for wound age estimation related to cutaneous ecchymoses. Thanks to the collaboration with the Institute of Legal Medicine, University of Milan, the development of the present strategy is carried out on human autopsy skin samples that from an analytical point of view represent the most adequate and realistic model, compared to tissues from sacrificed animals or synthetic skin. Both ecchymotic wound tissues and uninjured skin tissues (reference/control samples) were collected.

O1 FC

Preliminary results on the development of a procedure for protein extraction from dermal-epidermal tissues with subcutaneous layer and on protein analysis using LC-high resolution mass spectrometry with data-dependent acquisition are here reported. Tissue disruption and homogenization prior protein extraction resulted the first challenging issue due to hard skin texture and very reduced dimensions of specimens. Moreover, the high heterogeneity of the investigated dermal-epidermal tissues with subcutaneous layer is another aspect to be addressed. For this purpose, different strategies, applied also in combination, were compared. After a defatting step with hexane, they involved the use of keratolytic agents, a stainless steel home-made mortar immersed in liquid nitrogen, beads-based tissue homogenization, and a sonicator immersion probe. Different protein extraction buffers, i.e. UTC and RIPA, were tested, extracting about 10 mg of proteins/g of skin tissue. Taking into account the presence of matrix interferents, detergent residues and highly abundant proteins, such as keratin, different protocols for proteolysis were applied involving a final purification step on C18 cartridges before analysis. The different approaches were evaluated in terms of the total protein extraction by Bradford assay, gel electrophoretic protein separation, matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) and LC-/high resolution MS analysis of the digested extracts. These results represent the starting point towards a broader and ambitious interdisciplinary research project aimed at developing a reliable and robust strategy for would age estimation based on the application of target LC-MS/MS method and on multivariate data processing.

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O2 FC

UPDATING PROCEDURES IN THANATOCHEMISTRY: A MULTIWAY ANALYTICAL PLATFORM FOR POST MORTEM INTERVAL (PMI) ESTIMATION IN VITREOUS HUMOR

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A novel multiway approach is proposed based on spectroscopy and thermogravimetry associated to chemometrics, able to provide a multiparametric characterization of vitreous humor as a function of the time since death. Postmortem examination of the body was performed on hospital deaths occurred in casualty by medico-legal authopsy in order to estimate the precise time since death. The ICP-OES analysis was used to determine micro and macro elements in vitreous specimen that were found to be diagnostic in predicting the Post Mortem Interval (PMI). The thermogravimetric outcomes revealed that the percentage of bulk and bound water may be correlated to the spectroscopic analysis and chemometric tools were used to compare results and to develop a model of prediction of PMI. A significant role of P, S and Mg in addition to the potassium concentration may be observed in determining the death interval. In addition, the multiway analytical platform permitted to increase in the accuracy ofPMI estimation with respect to conventional procedures and to extend the investigation of PMI to 15 days [1].

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O3 FC

DETERMINATION OF FENTANYL DERIVATIVES IN ORAL FLUID BY MEANS OF MEPS EXTRACTION FOLLOWED BY UHPLC-HRMS/MS

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In recent years, the synthesis and introduction into the illicit market of novel psychoactive substances (NPS) has reached alarming levels: more than 700 compounds have been identified by the European Monitoring Center for Drugs and Drug Addiction (EMCDDA) [1]. Among the newest NPS, synthetic opioids deserve special attention, in particular fentanyl derivatives that in 2018 covered more than 70% of the world demand for opioids. Fentanyls produce drowsiness and euphoria, the latter being less pronounced than with heroin and morphine. In 2018 thousands of fatal events were reported in USA [2]. As concern EU, the phenomenon of fentanyl-derivatives consumption is increasing, for example in Estonia, as many as 60 % of applicants for treatment services in 2009 reported fentanyl as their primary drug, and in Spain fentanyl analogues were identified in crypto market-sourced heroin products [3].
In this context it is of significant importance to develop suitable tools for the identification of the most recent NPS and assess their consumption.
The aim of our research is the development of innovative, fast and simple analytical methods, for the determination of these compounds in different biological matrices, both conventional such as urine and plasma and alternative such as oral fluids (OF). All these matrices are equally important for forensic purposes as they give different kind of information about the time of assumption and they show some distinctive features.
A method of pretreatment for OF has been developed by using Microextraction on packed sorbent (MEPS), a novel technique which is based on the miniaturization of SPE. This novel microextraction technique has demonstrated to be suitable for OF handling, allowing the use of a small volume of sample (100 µL) which is a critical issue in OF testing [4]. The developed method allows to effectively extract the analytes from the matrix, and to detect them with high sensitivity thanks to the high enrichment factor and the reduction of the matrix effect. In order to demonstrate its high versatility, the same technique will then be applied to the clean-up of plasma and urine [5].
An UHPLC-HRMS/MS method has been developed for 12 fentanyl-derivatives including 6 metabolites. Target analytes separation was performed by mean of a C18 Polar column which allows the elution of all compounds in 10 min.

O3 FC

Qualitative and quantitative analyses were carried out by means of an high-resolution mass spectrometer with Orbitrap technology equipped with an H-ESI source operating in positive ionization mode. Acquisition mode was data dependent, which consent to perform the identification and the quantitation of all target analytes in a single chromatographic run, comparing retention times, fragmentation spectra and peak areas with an analytical standard. At the same time retrospective data analysis of the analyzed samples would be possible for the potential identification of unknowns. The analytical method was validated according to international guidelines [6].

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O4 FC

FACTORS AFFECTING THE VARIABILITY OF BREATH COMPOSITION

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Breath analysis is considered a promising tool for the non-invasive monitoring of health conditions relying on the almost instantaneous equilibrium of volatile chemicals in blood and lung air across the alveolar-capillary membranes. Breath composition contains information concerning the present and past exposure to xenobiotics, food and beverage consumption, normal and abnormal physiology as well as the presence of bacteria. Such richness of information becomes a limitation when the purpose is to diagnose a pathology, as intra- and inter-individual variabilities hinder the classification of subjects into healthy or pathologic and the identification of specific biomarkers or "breathprints". In addition, the lack of standardized procedures for breath sampling and analysis makes the comparison of results obtained from different research groups complicated.
For these reasons, in this study we tried to assess how factors such as sampling conditions, circadian rhythms and diet affect the inter- and intra-individual variability. The assessment of such variability and the weight of the different factors is important to evaluate the number of subjects to enrole and the statistical power of tests using breath biomarkers to distinguish patients and nominally healthy individuals. Breath composition was analysed in 20 healthy subjects following an omnivorous (n= 10) or a vegan (n= 10) diet under different conditions. After acquaintance of the subject with the sampling device, during which the CO_2 profile in breath was acquired, 25 mL of mixed breath were collected at 15 mL/min into needle trap devices loaded with an internal standard. Volatile organic compounds (VOCs) were analysed by needle trap micro-extraction coupled to gas chromatography tandem mass spectrometry. The chromatographic separation was carried out by a DB-624 ultra-inert capillary column and the mass spectrometer was operated in multiple reaction monitoring (MRM) mode. Qualifier and quantifier transitions were monitored for each compound.
The experiments showed that the effect of respiratory rate and ventilation on the composition of breath differs among VOCs, where the largest variations were observed with isoprene and acetone. Vegans showed lower values of VOCs related to oxidative stress compared to omnivorous subjects, but differences were not very large and a higher number of volunteers is needed for significance. Individual behaviours concerning smoke or consumption of specific foods were also mirrored in breath. Additional experiments, performed in the framework of a multicentre study organized from the breath community to compare different measurement approaches (Peppermint experiments), showed the individual variability in the washout of menthol after the ingestion of a peppermint.

O1 CHEM1

CHARACTERIZATION OF BASE OILS FOR ENGINE LUBRICANTS BY NIR AND FLUORESCENCE SPECTROSCOPIES COUPLED WITH CHEMOMETRICS

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The present study is focused on Engine Lubricant also called Engine Oil or Motor Oil.
Typically, lubricants contain 80% base oil and less than 20% additives that improve the oil performances.
One of the most common classifications for lubricants is by the constituent base oil: mineral, synthetic or vegetable. Mineral oils are derived from crude oil, synthetics are man-made through a synthesizing process, while vegetable base oils, which are derived from plant oils, represent a very small percentage and are used primarily for renewable and environmental interests.
Although, generally, lubricants are based on one type of base oil, mixtures of base oils are also used to meet performance requirements and to improve different properties in the formulations.
The American Petroleum Institute (API) has categorized base oils into five categories. The first three groups are refined from petroleum crude oil. Group IV base oils are full synthetic (polyalphaolefin) oils. Group V includes all other base oils not included in Groups I to IV.
It is possible to distinguish pure base oils by looking at the combination of physical properties such as viscosity index, density, colour, flash point, pour point, aniline point, thermal stability. Nevertheless, identification of a mixture of synthetic and mineral oils represents a big analytical challenge, due to the variable composition of base stock and additives.
A rapid solution to determine the type of base oil in lubricants could help the formulators when developing a new or tailored lubricant, targeting a given performance level. Since spectroscopy techniques are low-cost, green, non-destructive and fast, in order to reach this goal, the capabilities of near infrared (NIR) and Excitation-Emission matrix (EEM) fluorescence spectroscopies coupled with chemometrics have been investigated.
Fifty-three base oil samples and 25 lubricant samples have been analyzed by means of NIR and fluorescence spectroscopy. NIR spectra were acquired with a FT-NIR spectrophotometer (Buchi NIRFlex N-500), in the 4000-10000 cm^{-1} range at 4 cm^{-1} resolution. All the experiments were performed at controlled temperature (35°C). The Excitation–Emission fluorescence measurements were performed at room temperature with a PerkinElmer LS 55 spectrometer. According to the results of a preliminary D-Optimal experimental design, the excitation spectra were recorded between 200 and 500 nm, the emission wavelengths ranged from 300 to 900 nm; the excitation and emission monochromator slits were set to 4.5 and 11.0 nm, respectively, and the scan speed was set at 200 nm/min.

O1 CHEM1

Principal Component Analysis (PCA) was performed as a multivariate display method in order to visualize the data structure, and multivariate classification tools were investigated in order to distinguish among different API base oil groups.
Both NIR and fluorescence results showed a potential for differentiating the different base oil samples and their mixtures according to the API categories. Spectroscopic techniques appeared to be rapid and non-destructive analytical methods for the characterization of base oils into Engine Lubricants and, therefore, might also represent a promising tool for Gasoline Engine Oil analysis.

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O2 CHEM1

LINKING LINGUISTICS AND CHEMISTRY BY CHEMOMETRICS

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In the last decades, there has been a great development of data analysis tools suitable to cope with heterogeneous data, i.e. data which come from distinct sources and differ not only in their scales and values but as well in their structure, despite they relate to the same phenomena. A salient example is the integration of text description with numerical data for the same entities. This task requires, as a first step, extracting information from text documents and converting it to a suitable data format, which can then be handled by data analysis tools.
In the present work, we propose a Chemometrics data analysis pipeline to link text data with analytical data and we evaluate it in the applicative context of food consumption and consumer preference/expectation.
The consumers' interest in how food is produced and prepared has recently strongly increased. Consumers tend nowadays to be more aware about the different aspects regarding food consumption and, in line with this trend, new-concept restaurants, new food production techniques and experiments on recipes and food pairings are constantly developed. This phenomenon is driven by high-quality standards and often speaks a language based on what can be called the "craft rhetoric", for which craft/handmade is opposed to industrial, and mass production is opposed to artisanal [1].
Analytical chemistry in synergy with advanced data analysis can be profitably used to build new tools to aid consumers when choosing and pairing foodstuff, and producers to meet the consumers' expectations. In this perspective, the aim of the present study is to investigate the links between the "objective" world of analytical chemical profiling – e.g. using spectroscopy – and the "subjective" world of consumers tasting and describing food.
Consumer's preferences are traditionally assessed by directly interviewing small groups of people, but with the growth of the Internet and its web communities, mining online-posted reviews has become an interesting approach for assessing product appreciation and reception. Huge amounts of user-generated data are available today in very different formats, such as numeric scores, logical scores (in the form of like/dislike), geotags and written descriptions.
These shifts in how food is chosen and consumed, has led the beer industry towards massive changes, propelled by the explosion of craft and micro-breweries and the spread of home brewing. In relation to this, a data set about beer was used as a benchmark: spectroscopic data were previously acquired and analyzed by us [2], while user-generated reviews were

mined from the RateBeer website (https://www.ratebeer.com/), a sort of social network for beer enthusiasts.
The proposed Chemometric strategy comprises:
i) Text analysis methods [2] to process the user-generated reviews and convert them into numeric format, by the bag-of-words approach [3].
ii) Principal component analysis–generalized canonical analysis (PCA–GCA,[4]) to investigate the links between spectral and text data.
Moreover, to select subsets of terms from the text data two approaches were used: topics extraction using penalized matrix decomposition [5] and manually-defined sets of terms related to specific aspects of beer making and tasting.
Overall, twenty topics were identified and correlated with spectral features, a representative example, topic "Hops" is shown in Figure 1.

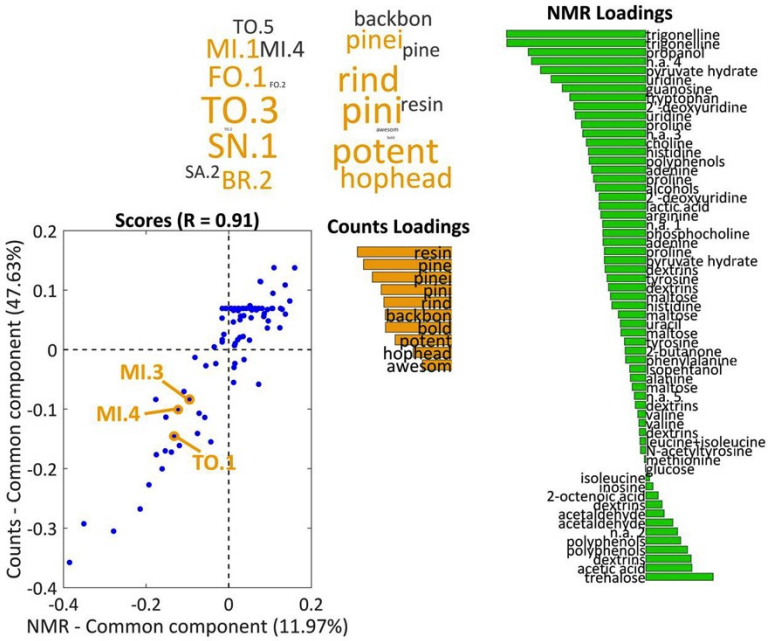

Figure 1. Results from PCA-GCA, on the bottom the scores of the common component , in the middle the loadings for text counts (orange) and for NMR features (green). On top the topic's characteristic terms and the main representative beer samples for this topic, represented as wordclouds.

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O3 CHEM1

CHEDDAR CHEESE RIPENING STUDY BY LOW-LEVEL DATA FUSION AND ANOVA-SIMULTANEOUS COMPONENT ANALYSIS

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In general, cheese varieties can be divided into two macro-categories: fresh cheeses, which are generally acid-coagulated and a little rennet-coagulated, and mature/ripened cheeses, which are rennet-coagulated. In the latter case, the production process is divided into two steps: the manufacturing step and the ripening, which approximately lasts between two weeks and two years, during which characteristic flavor and texture develop.
Among dairy products, Cheddar is one of the most exported by the European Union; for this reason, the quality control of this commodity has gained importance, especially during the phase of ripening, which directly affects the product outcome due to the possible appearance of unwanted aromas and flavors.
In literature, several methods were proposed to evaluate the ageing of cheese and its effects, but, despite most of them exploit very performing analytical techniques, they are also destructive and/or time-consuming, as in the case of Gas Chromatography–Mass Spectrometry (GC–MS), Enzyme-Linked Immunosorbent Assay (ELISA) and others. In addition, to better understand a chemical process that evolves over time, such as ageing/ripening, a non-destructive and rapid technique would be the most suitable solution for industrial purposes. For this reason, spectroscopy coupled to chemometrics is becoming the best choice for addressing this kind of problem. In particular, ANOVA-Simultaneous Component Analysis (ASCA) [1] combines the experimental design with the multivariate data analysis and this allows evaluating by an exploratory analysis whether a factor in the process under study is significant and, in case, its effect, and also to identify possible interactions among factors.
Furthermore, the possibility of working with many analytical instruments/facilities increased in the last years, so it has become frequent to handle multi-platform data sets. In principle, the diverse data blocks could be individually examined, but it has been demonstrated that handling them by means of data fusion strategies, could increase their performances. Despite multi-block analysis has been widely explored in many contexts (such as in classification or regression problems) [2], in literature, so far, there are no applications in the ASCA context.
Under this perspective, the purpose of this study is evaluating how two factors (*i.e.* ageing time and storage temperature) and their interaction affect the Cheddar cheese ripening by means of Raman and Mid-Infrared (MIR) spectroscopies coupled to ASCA in the framework

of a Low-Level data fusion strategy, which represents a novel approach both from the dairy product analysis and multi-block analysis point of views.
Raman and MIR spectroscopies were chosen because they provide complementary information about the matrix under study and, moreover, they exhibit the advantages of being non-destructive and less expensive and time-consuming with respect to the techniques commonly used in food quality control procedures.
As mentioned above, the first factor (F1) was considered in the study is the storage of the samples; half of them were kept at fridge temperature (4°C), and half of them were kept at room temperature (25°C) in order to evaluate its influence on their ageing. As already mentioned, the second factor (F2) which was taken into account is the ripening time; measurements were carried out within two weeks at a distance of about two days from each other both by means of Raman and MIR spectroscopies. Eventually, their interaction (F12) was evaluated and, in order to have a more comprehensive point of view, the F2 and F12 matrices were modeled together (*i.e.* the corresponding matrices were summed) to highlight not only the average effect of the time, but also how the storage condition differently affect the process of ageing over time.

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O1 AS1

THE CONTRIBUTION OF XPS TO THE UNDERSTANDING OF THE FUNCTIONAL PROPERTIES OF TITANIUM DIOXIDE

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The need to enhance specific functional properties of titanium dioxide, for instance to extend the photoresponsivity to visible light and to increase the photocatalytic efficiency, leads to different strategies for the modification of its electronic structure. The generation of point defects in the structure of TiO_2, producing sub stoichiometric oxides (TiO_{2-x}) and the conjugation with carbon or C-based materials are two convenient ways to modify the electronic features of TiO_2 [1]. In this work the X-ray photoelectron spectroscopy (XPS) was exploited for investigating TiO_{2-x} materials synthesized starting from hybrid chemical gels in which titanium is involved in a charge transfer complexation equilibrium with acetylacetonate and annealed in different environments, in order to identify the species responsible for their catalytic properties. XPS has proved to be a powerful technique for this scope. It provides evidence of the formation of the TiO_2 – acetylacetonate complex by C 1s photoelectron peak. Ti^{3+} and sub-oxides were clearly identified in the Ti 2p peaks of the samples calcined for 1h in a tubular furnace with the ends of the quartz tube open to air (static air conditions) (Figure 1). The extraordinary high concentration of Ti atoms with oxidation states lower than IV (about 26%) justify the unusual light absorption in the entire visible range [2]. When the hybrid TiO_2 – acetylacetonate samples were annealed at 400°C in laminar airflow, under nitrogen atmosphere or under a hydrogen/nitrogen mixture, no reduced Ti^{3+} was observed (Figure 1). Despite the absence of reduced Ti species, these materials showed significant yields of H_2, even without a metal co-catalyst both under UV irradiation and visible light. Also in this case, XPS provides an effective justification of the materials' properties. A detailed curve fitting of C 1s XP-peaks, following the approach developed to interpret complex carbon spectra of particulate matter [3], allowed us to observe the presence of a component due to elemental carbon at 284.2 eV (Figure 1), whose content increases from 7% for the air-annealed samples to 11-12% for the samples heated in H_2/N_2 mixture. No elemental carbon was detected in the samples annealed under static air conditions.

O1 AS1

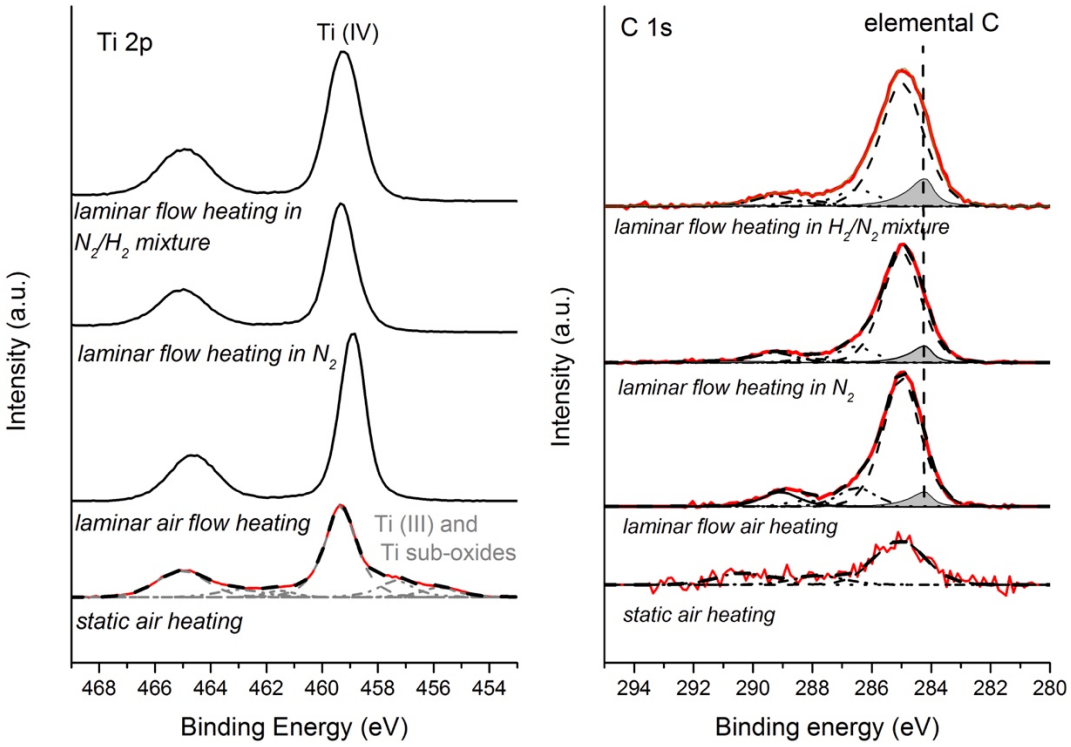

Figure 1. High-resolution XP- spectra of Ti 2p and C 1s acquired on samples heated at 400°C in various environments.

To clarify if the elemental carbon presence is due to C-doping of TiO_2, the XPS valence bands of the samples were also acquired and processed. The shape of the valence bands was typical for anatase for all the samples being the peak due to σ bonding O2p electrons more intense than the one due to π bonding. The binding energies of the valence band maxima, determined using the linear extraction method [4], were within the experimental error for all the samples, despite the different atmosphere of the thermal treatments. The absence of any extra electronic state above the valence band edge, which are supposed to be present in the case of C-doped TiO_2 [5] as well as the lack of any noticeable shift of XRD peaks lead us to exclude that carbon behaves as a dopant and supports the idea of the formation of graphitic carbon upon heating.

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O2 AS1

AN APPLICATION OF PARAFAC ON EXCITATION–EMISSION MATRIX FLUORESCENCE SPECTRA FOR GREEN TEA CHARACTERISATION

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The main objective of the present work was to evaluate the potential use of excitation–emission matrix (EEM) fluorescence spectroscopy, combined with a multi-way decomposition method such as Parallel Factor Analysis (PARAFAC) [1], for the characterization of green tea (GT) samples.
The claimed beneficial effects of GT are ascribed to catechins and methylxanthines, used as not only as chemical descriptors but as indicators of the geographical origin of tea.
The three-dimensional spectra of 50 GT samples (22 Japanese and 28 Chinese) were recorded using a Perkin–Elmer LS55 (Perkin-Elmer Ltd., Beaconsfield, UK) fluorescence spectrometer, a standard cell holder and a 10 mm quartz SUPRASIL® cell; excitation spectra were recorded between 200 and 290 nm, whereas the emission wavelengths ranged from 295 to 800 nm. The use of PARAFAC allowed to decompose the three-way arrays in EEM data into trilinear components and to detect the main fluorophores in GT samples; in particular, in the second mode of PARAFAC, four signals were highlighted that can be interpreted as the emission spectra of four tentative fluorophores.

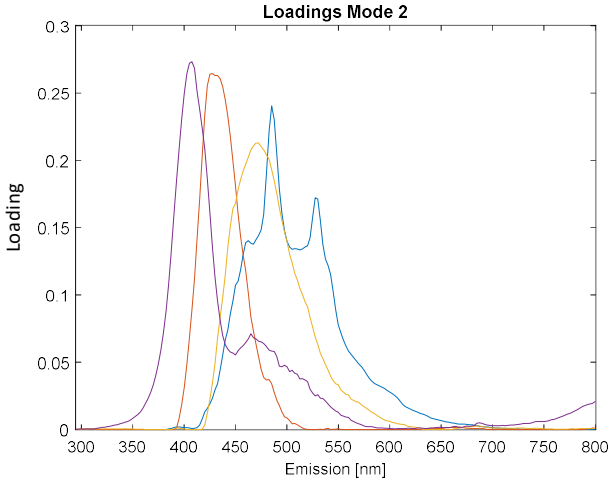

Figure 1. PARAFAC results: the profiles show the loadings on the second mode (emission profiles)

O2 AS1

The emission bands at 400-450 nm are due to fluorophores particularly present in Chinese green teas and that the broad band at 500-550 nm is related to the presence of chemical compounds more abundant in Japanese green teas.
The two bands (in violet and red) at 400-450 nm and the band with maximum around 470 nm (in yellow) correspond to the fluorescence emission of catechins and methylxanthines respectively, which are more abundant in Chinese samples and the broad band around 500-550nm is attributable to carotenoids which are recognized in particularly high quantities in Japanese tea. A cyclodextrin-modified micellar electrokinetic chromatography method was employed to quantify the most represented catechins and methylxanthines in GT samples and the outcomes were in agreement with the fluorescence spectroscopy observations.
This study shows that EEM fluorescence spectroscopy combined with chemometrics offers a promising approach for the discrimination of green tea samples based on their geographical origins.

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O3 AS1

REVERSIBLE COLORIMETRIC ARRAY FOR pH DETERMINATION IN AQUEOUS SOLUTIONS AND IN VAPOURS

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Recently we deposited a patent of a colorimetric array, designed to follow the degradation process of meat samples. [1]
For this purpose, in a previous stage, we selected a panel of commercially available dyes, mostly characterized by different pKa values [2]. Consequently, the indicators, fixed on the solid membrane, change their colours, into a range around neutrality, as function of the pH of the headspace over meat samples. We demonstrated that the evolution of the change of dyes is correlated to different degradation steps.
Nevertheless, in the view of in-field application, more recently, we were forced to change the support so that the indicators were covalently bound into a suitable polymeric support, with well-defined plastic properties. For this purpose, we chose ethylene–vinyl alcohol copolymers, EVOH, a polymer commonly used in food packaging. The synthesis was optimized, and the products obtained by synthesis were characterized from chemical-physical and analytical points of view, performing DSC, IR, EDX, UV-Vis; a colour analysis, based on RGB triplets, was conducted. In figure 1, some of the raw materials, as obtained in the last step of the synthesis (in the beakers) are presented. In the same photo, just below, the extruded films, cut in small squares, after equilibration at different pH values are shown.

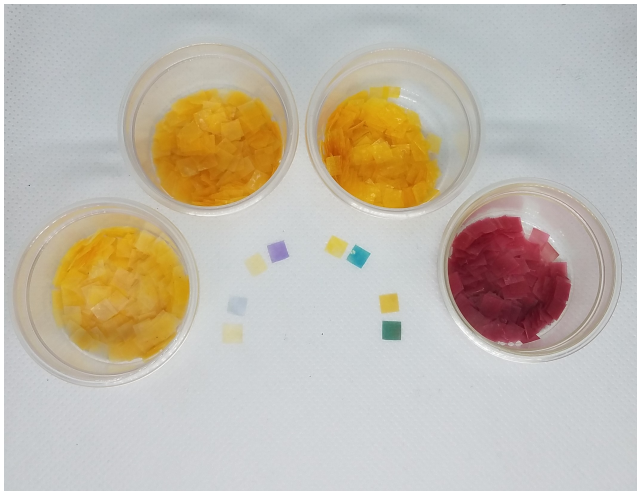

Figure 1. Some example of the products obtained from polymerization reaction with dyes.

Because of the covalent anchorage of dyes, differently from what already experimented when the same molecules were fixed on an ion exchange membrane, we observed a shift of

pKa values toward the basic region of around one log unit. This finding was common in all the polymeric films so long produced.
As a consequence the final array for meat spoilage sensing must be modified accordingly, taking this evidence into account. The modification is possible, since in the preliminary part of the study we employed a large number of candidate dyes with similar, but sufficiently different, pka values, so that the most appropriate ones, suitable to our primary purpose, can be re-selected.
As a collateral effect of this evidence, here in we explore the possible employment of the new polymeric array in a wider application, respect to the "intelligent label", as it could be the case of litmus test. Indeed, such a device is not at all new, but the array, here still presented as a very prelaminar prototype, is not only cheap but has the advantage to be reversible and suitable for solution and vapours.
The preliminary results are shown. The signals are the RBG indexes, the response the pH of the solutions, the PLS the tool employed to model the data set. Particular care is reserved for assessing the true pH values to the solutions contacted with the devices. For the more acid region, the pH assigned to the solution can be obtained by the total mineral acid, directly titrated, but the same cannot be done for the neutral region where we were forced to employ buffer solutions. In this case the true value, instead of depending on the choice of log k values of the weak acid, is measured with the glass electrode.
As it was and still is for the food application, also in this case the final goal is to have the reference colours for a naked eye detection that allow attributing the correct pH values to unknown samples. We want, if not now in the near future, to explore the different in-field applications, the advantage coming from an easy and reliable reuse, and the the possibility of assessing quickly the pH of vapours as shown by preliminary tests.

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O1 SS2

PREPARATIVE MULTIDIMENSIONAL GAS CHROMATOGRAPHY COUPLED TO SPECTROSCOPIC ANALYSIS AS A POWERFUL APPROACH FOR THE ISOLATION AND CHARACTERIZATION OF UNKNOWN MOLECULES

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The growing interest towards new molecules, is leading researchers and industries to investigate natural matrices. The identification of unknown components, requires the isolation of the target analytes with a high purity degree. Conventional GC analysis for preparative purpose presents different issues when highly pure compounds have to be collected at milligrams level in a reasonable time. A limited amount of neat or diluted sample can be analyzed in each run due to the GC column sample capacity and efficiency. The total analysis time is greatly affected by the sample injection volume. Moreover, the purity degree of the collected fraction is often unsatisfactory due to the presence of coeluted compounds. In order to provide an enhanced sample capacity, wide-bore columns (0.53 mm I.D.) are commonly used, but an excess of on-column sample amount results in overloaded peaks and decreased resolution. Aiming to reach an improvement in terms of both efficiency and resolution, a three-dimensional GC-Prep system equipped with three Deans switch transfer devices was exploited. Different stationary phases were used, providing an orthogonal selectivity for peak purification. The third GC column outlet was connected to a lab-made collection system, which allowed an easy isolation of the target volatile compounds, by means of their re-condensation into a quartz tube. The potentiality of such a heart-cutting multidimensional approach, combined with spectroscopic analysis, provides a useful starting point for the identification of possible highly valuable molecules for industrial and biological evaluations.

O2 SS2

MOLECULAR FINGERPRINTING OF TRADITIONAL FOOD PRODUCTS BY ULTRA-HIGH RESOLUTION ESI-FT-ICR MASS SPECTROMETRY

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Mass spectral characterization of food materials has advanced rapidly in the past few years due in large part to the development and now routine availability of electrospray ionization (ESI) [1]. However, it is now apparent that food products exist as such complex mixtures that Ultra-High resolution electrospray ionization Fourier transform—ion cyclotron resonance mass spectrometry (ESI FT-ICR MS) at high magnetic fields is currently the only technique capable of resolving individual molecules [2]. In this work, a Mass Spectrometry-based phytochemical screening was performed on several traditional food products produced in the Basilicata region (Italy). Ultra-High Resolution ESI-FT-ICR Mass Spectrometry data obtained from food sample analysis were used to perform a rapid analysis of metabolome by converting accurate m/z values in putative elemental formulae. Molecular formula maps, or molecular fingerprints, were obtained by making 2D Van Krevelen plots, that lead to a direct identification of different classes of metabolites [3]. The presence of important metabolite classes, i.e. fatty acid derivatives, tannins, amino acids and peptides, carbohydrates and polyphenolic derivatives, was assessed. Moreover, differences among Van Krevelen plots could be noticed from their direct comparison, thus reflecting differences in promoted biochemical pathways and suggesting the presence of biomarkers, that can eventually be identified by a target approach. Thus, molecular fingerprints prove to be an innovative tool, unique and full informative about food product metabolic content, that could be useful for food authentication and traceability.

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O3 SS2

ADVANCED PREPARATIVE APPROACHES FOR THE COLLECTION AND STRUCTURE ELUCIDATION OF VOLATILE COMPONENTS FROM COMPLEX SAMPLES

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The collection of analytes from natural sources is the goal of each preparative system. Conventional GC analysis for preparative purpose presents different limitations: although wide-bore columns (0.53 mm I.D.) are commonly used an excess of on-column sample amounts could result in skewed peaks and decreased resolution. On the other hand, the collection of pure components requires the injection of lower amounts in order to avoid coelutions on the wide-bore column. The higher is the injection volume, the lower is the total time required to collect a specific compound, thus the highest injection volume should be always used. Aiming to improve the productivity of the system a multidimensional prep-GC instrument is presented with the goal to reduce the total collection time and to improve the purity of the components collected. A prep-MDGC system was successfully used for the collection of pure components ranging from 10 to 30% concentration, collected at the milligram level, to allow a further characterization by means of other techniques (NMR, FTIR, MS). The system consists of an SLB-5ms - Supelcowax 10 - SLB-IL59 ionic liquid stationary phase (0.53 I.D.) combination used in the three GC dimensions, in order to provide three distinct selectivities. A preparative station, connected at the 3rd GC column outlet, allowed the re-condensation of pure components in a tube. The demands for the collection analytes at concentrations <10%, would consist in an increased sample injection volume, but this option could lead to the exceed the GC liner capacity. To improve the capability of the system, an on-line 4D chromatographic system (prep LC-GC-GC-GC) instrument can be adopted enabling the injection of higher sample volumes, the reduction of collection times, while maintaining high levels of purity. The system can be operated in different configurations, based on the complexity of the sample, exploiting a front-end LC pre-separation (whenever required by the complexity of the sample) before the three GC dimensions. Different applications are reported describing the potentiality of such an approach to provide a tool for the identification of possible valuable molecules for industrial and biological evaluations.

O1 CHEM2

A UNIFIED SIMCA FRAMEWORK FOR SINGLE AND MULTI-BLOCK DATA

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Modeling classification techniques, sometimes also called one-class classifiers, have several advantages over discriminant ones, especially when dealing with asymmetric problems, where there is only one category of interest [1]. Indeed, in class modeling, attention is focused on a single category at the time, whose class space is built only on the basis of the data collected on samples from that particular group. Classification is then carried out as an outlier detection problem: if a sample is found to be an outlier with respect to the class model (usually, according to a distance to the model criterion), is predicted as not belonging to the category under exam. Among the methods available in the literature for class modeling, soft independent modeling of class analogies (SIMCA) [2] is by far the most commonly used. In SIMCA, the systematic variability among samples belonging to the investigated category is captured by a principal component model of appropriate dimensionality so that the classification of unknown individuals is based on the definition of a distance to the model, which is calculated by combining residuals with a distance in the scores space, which is usually Mahalanobis-like.
However, when dealing with irregularly dispersed or, in general, moderately to highly heterogeneous classes, this may result in a shape of the model class space not corresponding to the actual one, so that high sensitivity can be achieved only at the price of low specificity and vice versa. In such situations, the use of a recently developed ROC-based approach to fine tune the classification thresholds [3] can help in finding the best model efficiency, but further improvements may be expected by redefining the way the class space itself is calculated.
In the present communication, the possibility of defining the scores distribution non-parametrically by means of a gaussian mixture model (potential functions) is presented. Gaussian mixture models approximate the probability density function of a distribution of samples as a linear combination of basis functions (normally triangular or gaussian), centred on the measured points:

$$f(\boldsymbol{x}) = \sum_{i=1}^{N} c_i g(\boldsymbol{x} - \boldsymbol{x}_i)$$

$g(\boldsymbol{x} - \boldsymbol{x}_i)$ being a basis function centered in $\boldsymbol{x}_i$ and c_i the associated weight.
Such approach allows a more-tailored definition of the class space even in the case of severe deviations of the distribution of the class scores from normality, as shown in Figure 1, where the probability density function for the distribution of a set of scores on the first two principal components in a toy examples is shown. Class modeling is accomplished by identifying a threshold value of the potential (probability density function, $f(\boldsymbol{x})$), enclosing a fixed volume of the distribution, which is usually 95%. In practice, the estimation of this

threshold value is usually accomplished by sorting the potentials of the training samples and identifying the desired percentile of the sorted values.

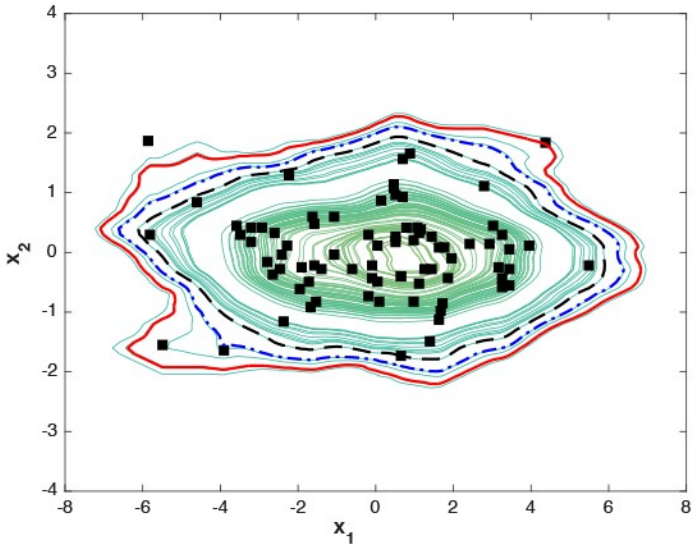

Figure 1. Use of potential functions to estimate the non-parametric scores distribution for a two component PC model. The red line indicates the threshold potential value for class acceptance (95th percentile of the distribution).

Due to its properties, this approach can easily be extended to the multi-block case in a framework which could be defined of mid-level data fusion, and could be applied on the scores calculated by means of different component models, not exclusively PCA.
The potential of the proposed approach will be illustrated by different examples involving food authentication both for the single- and the multi-block implementation.

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O2 CHEM2

CHOOSING THE CORRECT CHEMOMETRIC STRATEGY FOR QUALITY CONTROL AND AUTHENTICITY VERIFICATION: CONSOLIDATED APPROACHES AND RECENT TRENDS

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Qualitative data modelling is a fundamental branch of pattern recognition, with many applications in analytical chemistry, and embraces two main families: discriminant and class-modelling methods. The first strategy is appropriate when at least two classes are meaningfully defined in the problem under study, while the second strategy is the right choice when the focus is on a single class. For this reason, class-modelling methods are also referred to as one-class classifiers.

When quality or authenticity of a product have to be verified, the problem is often asymmetric, meaning that a class of interest (the one of the target product) has to be characterised against the rest of the world (i.e., the non-compliant samples). In these cases, since the interest is actually on a single target class (and the other class is not properly defined), class-modelling is the correct choice.

Nevertheless, discriminant approaches are very frequently applied for this purpose, claiming that class-modelling is less efficient in terms of model performances.

In the last years, some modified strategies that make use of discriminant methods for addressing asymmetric problems in a declared “one-class way” have been proposed. Such strategies define a dummy class, which can arise either from real measurements on dummy samples (blanks, solvents) or from random artificial calculations. Discriminant methods – including linear discriminant analysis (LDA) and partial least squares discriminant analysis (PLS-DA) – are then applied to discriminate the target class from the dummy class.

In the present study, these novel approaches are evaluated in depth, on real and simulated analytical datasets, and critically compared with standard class-modelling strategies, verifying whether they actually represent or not a reliable and more efficient alternative for addressing authentication problems.

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O3 CHEM2

A SOURCE APPORTIONMENT EXERCISE OF A SMALL DATASET OF PM10 AND PM2.5 USING POSITIVE MATRIX FACTORIZATION IN A RURAL SITE IN SOUTH ITALY

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Airborne Particulate Matter (APM) is used by the World Health Organization (WHO) as a proxy indicator for ambient air pollution. For this reason the identification as well the quantification of the different sources influencing PM levels at a receptor are essential for the estimation and improvement of Air Quality. The Source Apportionment at receptor site is reliable using receptor modelling techniques [1, 2]. Receptor models, relying on multivariate statistical methods (such as Positive Matrix Factorization – PMF), require the inputting of a matrix composed of a relatively large number of samples to function correctly [3]. A number of publications have sought to define objective criteria aimed at establishing the minimum number of samples for use with these receptor models. However, the issue is far from settled. Results of a source apportionment exercise using positive matrix factorization (PMF) for PM10 and PM2.5 are reported. This study is based on a small dataset of 29 PM10 and 33 PM2.5 samples for a receptor in a rural setup in Apulia (Southern Italy). Running PMF on the two size fractions separately resulted in the model not functioning correctly. We therefore, augmented the size of the dataset by aggregating the PM10 and PM2.5 data. The 5 factor solution obtained for the aggregated data was fairly rotationally stable, and was further refined by the rotational tools included in USEPA PMF v5. These refinements include the application of constraints on the solution, based on our knowledge of the chemical composition of the aerosol sources affecting the receptor. Additionally, the uncertainties associated with this solution were fully characterised using the improved error estimation techniques in USEPA PMF v5. The results of the error estimation techniques in PMF (BS, DISP and BS – DISP) show reasonable uncertainties in the tracer species. Five factors in all were isolated by PMF: ammonium sulfate, sea salt, mixed carbonaceous aerosol, crustal/Saharan dust and total traffic. These results obtained by PMF were further tested *inter alia*, by comparing them to those obtained by two other receptor modelling techniques: constrained weighted non-negative matrix factorization (CW – NMF) and chemical mass balance (CMB). The PMF results were confirmed by CW – NMF, while the

O3 CHEM2

CMB estimates show some explainable deviations in PM10 and in PM2.5. The results of this comparison suggest that the solution obtained by PMF is valid, indicating that for this particular airshed PMF managed to extract most of the information about the aerosol sources affecting the receptor – even from a dataset with a limited number of samples.

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O4 CHEM2

CHEMOMETRICS COMBINED WITH UNTARGETED MASS SPECTROMETRY FOR THE STUDY OF SAFFRON ADULTERATIONS

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Saffron (*Crocus Sativus L.*) is one of the most expensive spices in the world, due to its particular flavor, its limited areas of production and the laborious process required to obtain the final product. Therefore, it can be subject to fraud, as for example the undeclared addition of a cheaper spice, with the aim of illegally increase the seller's gain.
The aim of the present work is to develop an analytical method able to detect an addition of turmeric, safflower, marigold, or garlic in a saffron sample. These spices are, generally, the most used for saffron adulteration.
We analyzed several samples of spices by mass spectrometry. However, instead of looking at specific compounds, perhaps separating them by chromatography, we injected the samples (properly prepared) directly into the mass spectrometer. A single quadrupole spectrometer was used and the whole mass spectra of the samples were used for the following chemometric procedure. By a Principal Component Analysis, the most discriminative mass peaks for each adulterant spice were identified. In this way, the untargeted method can also support the targeted analysis, by confirming the presence of known discriminant molecules and adding new interesting analytes. Then, some saffron samples were manually adulterated with a known amount of each spice, and the mass spectra were used to quantify the added spice. This task was carried out by a multivariate standard addition method combined with the Net Analyte Signal (NAS) procedure [1].
Good results were obtained, thus encouraging the possibility to apply it to routine analyses in food authenticity control, also for other food matrices. Moreover, the untargeted single-quadrupole mass spectrometry was used to identify the presence of adulterants in saffron both qualitatively and quantitatively.

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O5 CHEM2

EFFECTIVE VALIDATION OF CHROMATOGRAPHIC ANALYTICAL METHODS

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The validation of an analytical method is mandatory to assess its reliability. At the present, there is not an official validation procedure, despite several organizations and scientists have tried to standardize the protocol. According to the purpose of the analysis (e.g., qualitative, quantitative) and the application field, the evaluation of specific parameters is recommended [1,2]. For the validation of quantitative methods, a feature of utmost importance is represented by the calibration. Although most instrumental systems should theoretically supply a straight correlation between concentrations and analytical signals, in reality some interfering physical and chemical phenomena may result in a deviation from the expected linear trend and/or heterogeneous distribution of data-point at different concentration levels [3]. The occurrence of heteroscedasticity is responsible of the erroneous evaluation of other parameters, among which the limit of detection (LOD).
In our daily work with biological matrices, LC-MS/MS and GC-MS-based methods are continuously developed and/or updated to support the ongoing evolution of clinical and toxicological requirements. This changing scenario opens further analytical inquiries not addressed in standardized validation procedures, including practical questions that frequently need careful consideration, including (i) for how long is a calibration curve valid and reliable? (ii) Should it be tested before each working section? (iii) Is heteroscedasticity distributed along the whole range of calibration, or is it peculiar only of the highest portion of the calibration range? (iii) Is it sufficient to evaluate the validation parameters only at the lower, middle and upper levels or is it safer to consider all the calibration points? (iv) Whenever deviation from linearity occurs at the high concentration range, is it preferable to adopt a quadratic calibration model or to split the curve into two linear segments? To answer these questions, we developed an efficient and rigorous validation protocol. In practice, our approach consists in the systematic measurement of three replicates of the calibration curve in three different days. It allows the robust computation of the calibration curve (taking into consideration the heteroscedasticity and the contribution of the quadratic term), limit of detection (LOD), intra- and inter-day precision and accuracy, ion abundance repeatability, selectivity, specificity and carry over (Figure 1). Few further experiments are required for the evaluation of matrix effect, extraction recovery and retention time repeatability.
The computations are performed employing the R routine developed by Desharnais et al. [4,5] and in-home built Excel sheets.

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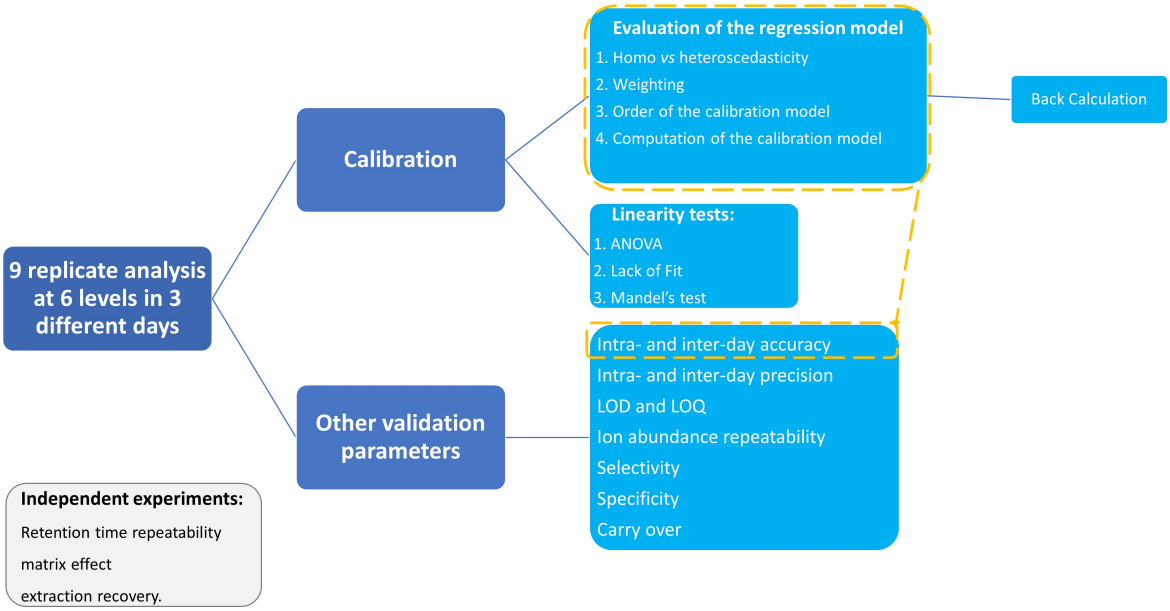

Figure 1. Scheme representing the validation protocol

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O6 CHEM2

QUALITY BY DESIGN APPROACH USED IN DEVELOPMENT OF NOVEL CRYSTALLINE FOOD PACKAGING MATERIALS

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Food waste is an issue of importance to global food security and good environmental governance, directly linked to environmental, economic and social impacts. In the EU, an estimated 20% of the total food produced is lost or wasted, while 43 million people cannot afford a quality meal every second day [1].
Currently, all the actors of the food chain (farmers, food manufacturers, retailers and consumers) are involved in preventing and reducing food waste. A way to reduce food waste can be related to the increase of the shelf-life of food as a result of improving the package type. An interesting approach to prevent food deterioration is the incorporation of antioxidants or antimicrobial agents in the packaging. Essential oils (EOs) are natural ingredients produced from plants with known antibacterial, antiviral, antifungal and insecticide properties [2] commonly used to this purpose. The aim of this study was to extend the use of natural products for packaging applications with cocrystallization. A further aim was to apply a Quality by Design (QbD) approach to optimize the inhibition of selected microorganisms involved in food deterioration. Even though cocrystals are largely known among the scientific community, their use has not systematically moved beyond pharmaceutical applications [3,4]. Cocrystals are multicomponent solid crystalline materials made by different chemical entities (i.e. the main ingredient and the coformer) with a given stoichiometric ratio. The presence of the conformer in the structure induces changes in the chemical environment of the EO in the solid providing a stable intermolecular network. The structure of a crystalline compound determines many fundamental physical properties of the material, and in the case of EOs, cocrystallization increases the melting point of the material thus inducing the stabilization of a liquid ingredient in a solid form. This is a really important matter since liquid or low-melting point compounds are not useful for industrial applications.
Our attention was focused on eugenol, carvacrol, cinnamaldehyde and thymol, which are mainly liquid at room temperature, thus requiring a way to stabilize them into the solid state to produce plastic films characterized by antimicrobial properties. New cocrystals were synthetized to tune the oil release profile at different environmental conditions (room temperature and refrigerated conditions), thus investigating their antibacterial properties

against microorganisms commonly detected in fruit and vegetables and comparing the achieved results with those of the pure oils.
Since data deriving from time kill assays (TKA) revealed that the required broad antimicrobial effect could be achieved only by using a mixture of the investigated EOs, a QbD approach was used to optimize the simultaneous inhibition of the selected microorganisms. QbD is a science- and risk-oriented quality paradigm [5] based on multivariate tools, which could facilitate process, product or method development and could lead to important advantages in terms of gained knowledge and risk management. QbD was applied for in-depth investigating the effect of critical parameters (CPs), represented by the concentration values of the selected EOs in a mixture, on critical attributes (CAs) related to the percentage of inhibition of the selected microorganisms. A Face Centered Design was used for estimating the coefficients of the quadratic models that correlate the CPs with the CAs; contour plots were drawn, allowing significant interactions and quadratic effects to be evidenced. A target value for the CAs was set and Monte-Carlo simulations were used to draw probability maps, showing how the CPs settings could be varied around a selected set-point, still guaranteeing an adequate level of probability that the desired requirements for all the CAs are fulfilled. This multidimensional optimum zone, identified as the Design Space, represents the core of QbD approach and is defined in terms of variation range of each CP under study. The design space was validated by a Plackett-Burman design and the obtained results showed that in every verification point the complete inhibition of all the considered bacteria was observed. Finally, the optimized packaging was characterized in terms of release of EOs and increased shelf-life of fruit and vegetable samples.

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O1 EAC2

DEVELOPMENT AND VALIDATION OF A UHPLC-MS/MS METHOD FOR THE IDENTIFICATION OF IRINOTECAN PHOTODEGRADATION PRODUCTS IN WATER SAMPLES

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Pharmaceuticals are one of the most relevant groups of emerging contaminants in aquatic environments due to their universal use, physicochemical properties and known mode of action in aquatic organisms at low concentrations [1]. Supported by the development of highly sensitive analytical and bioassay methods, the persistence and toxicity of anticancer drugs and their transformation products have been the focus of recent research on water pollution [2]. Irinotecan (CPT-11) is a water-soluble anticancer drug widely used to treat several types of cancer such as colon, small lung, ovarian, brain, gastric, cervical and pancreatic cancers. Studies have indicated that not all administered CPT-11 drug is metabolized, but an amount around 45-63% is excreted as parent drug by the human body [3] and enter the sewerage system ultimately reaching ground and surface waters. Even if the metabolites of CPT-11 are well-known and investigated, very limited information is present in the literature about the formation of photodegradation products that can naturally originate from sunlight irradiation when the drug is released in aqueous systems.
In the present study, CTP-11 solutions at 10.0 mg L^{-1} were irradiated for a maximum of 13 days by simulated sunlight through a solar box (Co.fo.me.gra 3000e, Milan, Italy) utilizing Xe lamp at 600 W m^{-2} and temperature of 35 °C. In the course of the photodegradation process, sample aliquots of about 3 mL were withdrawn after irradiation at prefixed time intervals. In order to monitor the progress of the photodegradation process, the sample aliquots were subsequently analysed by UV-Vis spectrophotometer (Jasco V-550, Milan, Italy). The intensity of CPT-11 decreased by 90% after 7.5 days of irradiation and no significant reduction of absorbance values was observed after 12 days.
A sensitive UHPLC-MS/MS method was developed employing a hybrid triple quadrupole/linear ion trap mass spectrometer (Nexera UHPLC-MS/MS, Shimadzu, Tokyo, and 3200 Qtrap, Sciex, Canada), that is able to work in data-dependent acquisition mode, in order to automatically obtain information about the unknown species formed by irradiation and to build a reaction monitoring method with the MS/MS fragmentation pattern of the species previously investigated. The method was validated obtaining for CPT-11 LOD and LOQ values of 0.02 and 0.05 ng mL^{-1}, respectively and MDL and MQL in river water of 0.03 and 0.10 ng mL^{-1}. Eight photodegradation products were identified and five of them for the first time. The total ion chromatogram (TIC) given in Fig. 1 shows the formation of the photodegradation products for the first five irradiation time points. The chemical structures of the unknown species formed in the photodegradation process were proposed on the basis of the following data: (i) the molecular mass identified by the quasi-molecular ion in

the MS, (ii) the isotopic pattern through the ER mode, (iii) the assignment of even or odd number of N atoms corresponding to an even or odd molecular mass, and (iv) the result of the MS/MS fragmentation analyses through the EPI mode. Hydrolysis experiments were also carried out on the same solutions preserved in the dark, but no formation of other species was highlighted. The method was applied to several real samples (river water, groundwater, well water before and after chlorination and samples collected close to a depurator outlet), but neither CPT-11 nor any of its photodegradation products were found. The outcomes of this study may be useful for updating the pollutant screening in water samples.

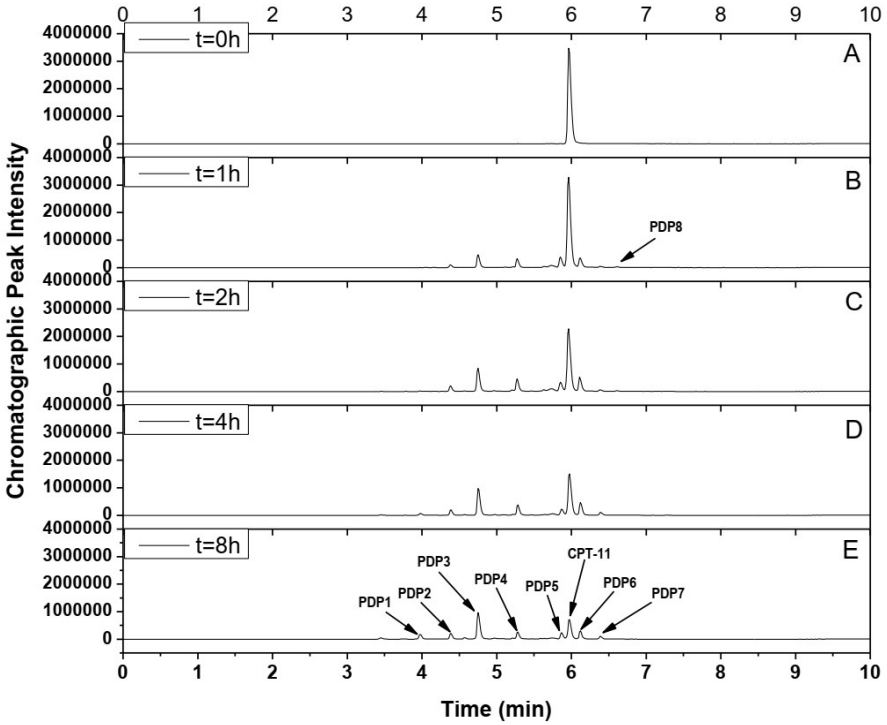

Figure 1.TIC for irradiated irinotecan solutions from time 0 to 8 h.

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O2 EAC2

DEVELOPMENT OF A NEW FRACTIONATION STRATEGY FOR THE ELEMENTAL AND ISOTOPIC ANALYSIS OF ATMOSPHERIC PARTICULATE COLLECTED AT THE ANTARCTIC PLATEAU

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The identification of the potential source areas (PSAs) of the atmospheric particulate collected at the Antarctic plateau would be a significant step forward to better understand the present changes in the southern hemisphere atmospheric circulation, as a consequence of the global change, and to improve the interpretation of ice core stratigraphies. To achieve this goal, in the framework of the National Antarctic Research Program, the project SIDDARTA planned to carry out physical, geochemical, mineralogic and isotopic measurements on aerosol, surface snow and snow-pit samples collected at Concordia Station (East Antarctica) in the 2017-2020 period, and to compare them with similar measurements performed on soil samples from South America and Australia as potential PSAs.
Preliminarily to this study, however, it was necessary to develop and validate a suitable analytical procedure for the elemental and isotopic analysis of the atmospheric particulate. In particular, a new sample fractionation scheme was designed to differentiate between the soluble and insoluble phases of the particulate and to properly pre-concentrate the analytes for the following instrumental analysis. The aerosol filters were treated in two ways: 1. by re-suspending the particles in ultra-pure water and concentrating insoluble compounds by filtration through a small surface area filter; 2. by re-suspending the aerosol content in ultra-pure-water and concentrate soluble and insoluble compounds, by sublimation, on a membrane with a small surface area [1]. The first approach is useful to compare the aerosol composition with ice core data, whereas the second approach can provide further information on relatively soluble components, such as carbonates and sulphates.
The procedure to obtain the total fraction involved an ultrasonic extraction of the particulate from the 90-mm Teflon filters using ultra-pure water in 50-mL polypropylene tubes. Then, the solutions were transferred into special Teflon tubes for the sublimation step. These tubes have a conical end where a suitable 20-mm membrane is located to collect the particulate matter after sublimation of the water. In this way, both soluble and non-soluble fractions are collected on a smaller surface of an optimal support for the direct analysis by proton-induced X-Ray emission (PIXE) [2] or laser ablation inductively coupled plasma mass

spectrometry (LA-ICP-MS) [3]. These membranes could also be treated by "wet chemistry" procedures, such as microwave-assisted acid digestion followed by elemental and isotopic analysis *via* inductively coupled plasma mass spectrometry (ICP-MS) techniques.
The entire procedure has been studied step by step to carefully evaluate the critical aspects that could negatively affect both blank levels and the analytical recovery. For this purpose, both ICP-MS and PIXE measurements were performed. The optimized method was then applied to analyze year-round samples of PM_{10} aerosol collected at Concordia Station, with monthly resolution. The target analytes were major and trace metals (including REEs) and isotopes of Sr and Pb for the provenance determination [4].

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O3 EAC2

HYDROCARBONS REMOVAL FROM BILGE WATER BY ADSORPTION ONTO ACTIVATED BIOCHAR FROM POSIDONIA OCEANICA

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The normal operations carried out on the boats during navigation generate waste waters such as oily bilge water. The latter is the aqueous mixture of potential pollutants of different origins and types: oily fluids, lubricants and greases, cleaning fluids and other wastes that accumulate in the lower part of the vessel [1,2]. The current legislation provides that they can be discharge directly into the sea if the concentrations of some components are below the expected limits. In particular, with regard to oil / hydrocarbons contamination, the current regulatory limit is 15 mg L^{-1} of total hydrocarbons. The present work starts from a public/private partnership funded by a grant of the Ministry of Economic Development (MiSE). Among the aims of the project, novel methods shall be tested for the reduction of hydrocarbons concentration at values below 5 mg L^{-1}. Moreover, instrumental techniques able to quickly measure the required low hydrocarbons concentration were tested. Among the different steps of bilge water treatment in pilot plant (coagulation, flotation, centrifugation, adsorption etc.), the latter requires the use of adsorbent materials able to reduce the oily concentration below the legal limits. Here we have chosen, optimized and tested materials obtained from bio-oil production waste, a biochar obtained by pyrolysis of *Posidonia oceanica*, a marine plant widespread in the Mediterranean sea.

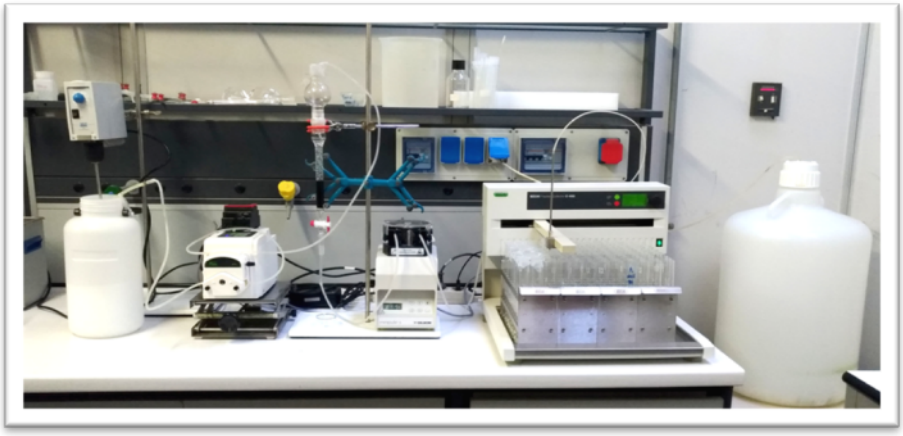

Figure 1. Benchtop pilot system for hydrocarbon/oil fraction removal from bilge water type dispersion.

The biochar has been characterized and adsorption experiments were carried out with the pristine biochar (not activated) and with two chemically activated biochars (BCB and BCA) by

means of acid or alkali treatments. Moreover, a commercial activated carbon (Filtrasorb 400) has been used for comparison purpose. Synthetic bilge waters were prepared following the reference standards [3] for the preparation of test fluids (used to test the bilge separator plant), containing DMA (distillate marine fuel) and SLS (sodium lauryl sulfate).
Batch adsorption isotherms were carried out without ionic medium and at different ionic strengths in NaCl in order to evaluate the effect of salinity on the adsorption ability of adsorbent materials. The same adsorbents were tested by column experiments. In particular, a bench pilot system was built (Figure 1.) and breakthrough curves were obtained changing amount of adsorbent material in column, flow rate, initial DMA and surfactant concentrations .
Several instrumental techniques (turbidimetry, TOC, HPLC-QQQ and HPLC-FLD) have been used to measure surfactant and hydrocarbon concentrations in experimental samples.
The batch experimental data were fitted with the most used isotherm models (Langmuir, Freundlich, Sips) and important considerations were made on the breakthrough curves of column experiments.

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O4 EAC2

ADVANCED OXIDATION PROCESSES (AOPs): INTERESTING SOLUTIONS FOR THE DEGRADATION OF EMERGING CONTAMINANTS IN REAL LIQUID PHASES

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Wastewater, as a result of the anthropic and industrial activities, contains many recalcitrant organic compounds such as pesticides, pharmaceuticals, surfactants, colouring matters and endocrine disrupting chemicals. In particular, the PPCPs (Pharmaceutical and Personal Care Products) have been detected in surface, ground and in drinking waters. Prolonged exposure over time of these substances, at low concentrations, can cause:

- allergies;
- development of antibiotic-resistance (antibiotics);
- effects on the endocrine system (hormone-acting drugs);
- cytolytic or cytostatic effects (antitumoral drugs).

All these represent a big problem for the health and for the environment, and are linked to the modern lifestyle [1]. People use many chemical-based products every day, which remain (as original or transformed compounds) in wastewater because the treatment plants (WWTP) were not designed to take out these chemicals. After that, these compounds end up in all liquid phases (lakes, rivers, sea) causing the problems described above.
Advanced oxidation processes (AOPs) have been proved as innovative and promising alternative route for the treatment of wastewater to destroy many kind of emerging pollutants [2].
This study examines the photocatalytic activity of titanium dioxide (TiO_2) towards removal of persistent organic pollutants (POPs) from water. Fluoroquinolones and benzodiazepines, antibiotics and psychoactive drugs respectively, commonly prescribed and used, have been selected as the object of study.
Two experiments were carried out using (i) TiO_2 as dispersed powder, and (ii) TiO_2 immobilized on borosilicate tubes (Figure 1). A cooled solar simulator equipped with a xenon lamp with 1,500 W total power, 500 W/m^2 irradiance, in the wavelength range 290-800 nm at 25°C constant temperature was used for sample irradiation.

O4 EAC2

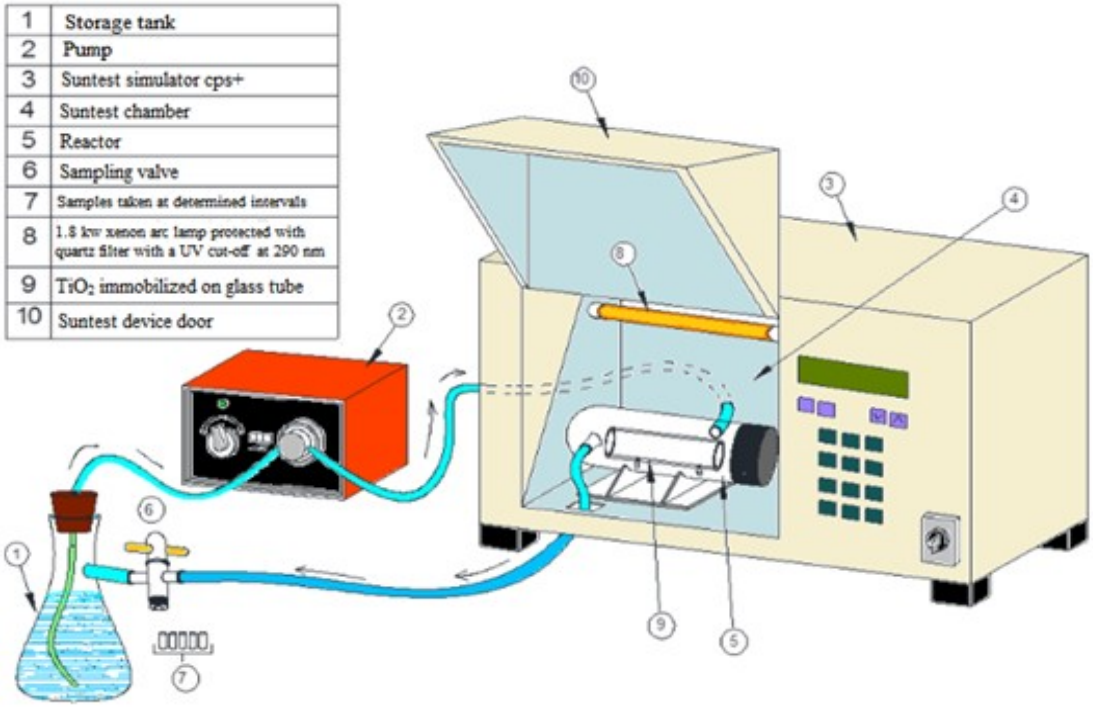

Figure 1. Cooled solar simulator equipped with TiO_2 immmobilized on glass tubes.

Kinetics of photoreactions were determined, and the identification of the photoproducts was performed using liquid chromatography coupled with micrOTOF-Q-II-Mass Spectrometer (LC-MS, Bruker Daltonik GmbH, Bremen).

The overall results suggest that active thin layer of TiO_2 immobilized on borosilicate surface can avoid the recovery problems related to the use of TiO_2 powder in heterogeneous photo-catalysis and may be a promising tool towards protecting the environment from emerging contaminants [3].

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O5 EAC2

POLYMER INCLUSION MEMBRANES (PIMs) AND SURFACE MODIFIED PIMs: CHARACTERIZATION AND OPTIMIZATION OF THE SEQUESTERING ABILITY TOWARDS Sn(II) BY A MULTI-ANALYTICAL APPROACH

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The employment of polymeric membranes to afford a wide range of energy- and environmental-related applications has attracted both research and industry. Among them, Polymer Inclusion Membranes (PIMs) have been investigated to produce materials with appealing properties in fields such as separation science, sensors, water treatment, while featuring cost competitiveness and ease of processability. PIMs are usually composed by a polymeric matrix, i.e. PVC or CTA (polyvinylchloride or cellulose triacetate), an extractant (a carrier) and a plasticizer and/or modifier. Such a simple chemistry may be finely tuned to gain membranes able to exert selective pollutants extractions with high efficiency, avoiding the environmental issues of organic solvents use. Bearing this in mind, this work has been focused on the synthesis of PIMs and surface modified PIMs able to exert sequestering properties towards Sn^{2+}. For this purpose, several PIMs were prepared, containing different amounts of PVC and CTA as polymeric matrix, Aliquat 336 or Alicy as plasticizers, and Thiomalic acid (SMAL) or montmorillonite modified with a thiolic group as extractants. In this last case, the use of a compatibilizing agent 3-aminopropyltriethoxysilane (APTES) was necessary to anchor the inorganic montmorillonite with the organic matrix of the polymer. The aim of this double approach is to obtain membranes where the extraction of the metal can be connected either to its diffusion within the bulk of the membrane or to a specific interaction with the functionalized surface. The sequestering ability of the PIMs was evaluated in a solution of Sn(II) 1 ppm by differential pulse – anodic stripping voltammetry. The PIMs was immersed directly into the electrochemical cell, thus allowing to collect a scan every two minutes and to profile the absorption kinetic in one hour. The composition of PIMs and the solution in which test their sequestering ability were selected with a D-Optimal experimental design. A total of 9 factors, 6 of which are 3-levels and 3 are 2-levels, while the maximum number of experiments to performed is 30. The candidate points submitted for the D-optimal design were planned on the basis of a fractional factorial design (1/12) and resulted to be 16.

O5 EAC2

Table 1. Factors influencing the sequestering ability of a PIM towards Sn^{2+} and their levels of variation.

Factors	**level -1**	**level 0**	**level +1**
ratio w/w polymer/additive	1	---	4
polymer	PVC	---	CTA
Ionic medium	NaCl	---	$NaNO_3$
additive	Alicy	no additive	Aliquat
sequestrant	SMAL	no sequestrant	MMT-SH
ratio w/w polymer/sequestrant	2	5	10
pH	2	3	4
surface area	2x2	2 x (1x2)	tea bag
Ionic strength	0	0.12	0.25

All the tested membranes were characterized by means of Thermogravimetric Analysis (TG-DTA), Differential Scanning Calorimetry (DSC), static contact angle measurements, tensile module, thickness and Raman investigations.

O6 EAC2

PORTABLE PHOTOREACTOR FOR A FAST ON-SITE MEASUREMENT OF THE PHOTOCATALYTIC ACTIVITY OF A MATERIAL

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The possible market for photocatalytic materials, in the form of powders, built-in powders, thin films and nano-structured materials, as well as devices directed to specific applications, is estimated in exponential growth. However, the central problem is the standardization of the procedures for photocatalytic efficiency evaluation of an illuminated catalyst. For gas/solid experiments different reactors, like batch or flow-through either continuous stirred-tank reactor (CSTR) or plug flow reactor (PFR), were proposed [1,2], in order to measure the rate of conversion of a standard substrate under controlled conditions. Experiments show that a CSTR configuration presents a lot of advantages for practical use, as any volume, any shape of catalyst and any flow of gas into the reactor can possibly be used [3]. In the framework of the SETNanoMetro EU project we developed and patented a portable CSTR photoreactor with controlled illumination and purposely designed fluidodynamics in order to obtain a photocatalytic rate evaluation independent on the measurement conditions, characteristic only of the catalyst [4]. The reactor allows fast (few minutes) and on site measurements with a choice of different substrates. This is a step in the direction to obtain a traceable measurement of photocatalytic activity and properly compare different catalysts.

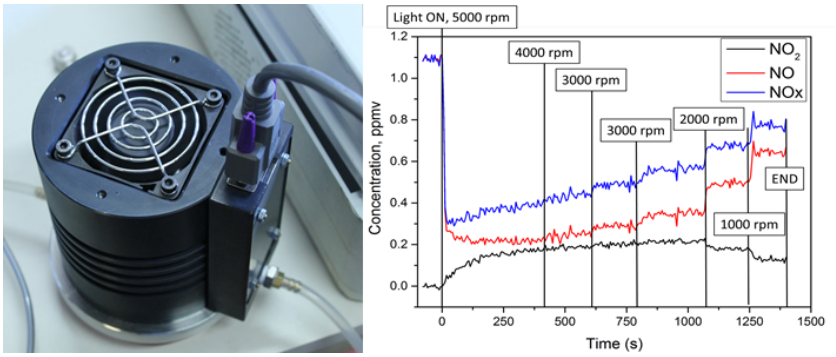

Figure 1. Portable photoreactor developed for photocatalytic activity evaluation and example of a test using NO as substrate

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O6 EAC2

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O1 SB2

INNOVATIVE PAPER-BASED ELECTROCHEMICAL SENSOR FOR MONITORING THE STATUS OF STEEL EMBEDDED IN CONCRETE STRUCTURES

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The use of reinforced concrete for the production of 20th Century buildings and artworks is one of the main key aspects of the buildings sector. The great interest that has focused the attention of building industry on reinforced concrete is due especially to its low costs and robustness. Indeed, concrete is an extraordinarily versatile construction material, which has been exploited in utilitarian, ornamental, and monumental structures during the last century. However, the artworks in concrete are subjected to several deterioration mechanisms including carbonation, freeze-thaw deterioration, corrosion of reinforcement, alkali silicate reaction, erosion of surface matrix, and superficial sulfate reaction [1-3]. The necessity to ensure the stability over the time of concrete-based buildings and artworks has been tragically highlighted within the last year, as a consequence of incidents caused by structural failure of important buildings, such as the collapse of Morandi's bridge, in Genoa (Italy). This makes essential to develop sustainable strategies and tools able to monitor the state of conservation of reinforced concrete-based buildings.
Herein, we present a miniaturized potentiometric sensor combined with a portable potentiostat for the easy, fast, and on-site monitoring of the state of conservation of reinforced concrete. The suitability of the sensor was demonstrated by analyzing several samples of reinforced concrete, prepared using same weights of Portland cement and river sand. The concrete reinforcements were simulated by embedding iron bars in the concrete samples. Additions of chloride and bicarbonate ions were carried out in the concrete samples in order to artificially induce degradation processes, such as corrosion of the iron bars and the decreasing of concrete's pH. To monitor the aging of such samples, the sensor is put in contact with the concrete surface and used as reference electrode, while the iron bar was used as working electrode. After having proved the working stability as well as the repeatability of the potentiometric measurement, the sensors were used to discriminate among the different concrete samples. Hence, we demonstrated that we are able to evaluate the ageing process before to see evident corrosion of the sample, by applying a potentiometric measurement is 30 s long.
Moreover, the developed potentiometric approach was tested on a real artwork seduta di Arman, 1973, in Milan (Italy). The application of the sensors on several parts of the artwork gave different measured potentials, in accordance with the different extent of exposition to aging conditions, and hence different corrosion levels. These results have confirmed the applicability and suitability of the sensor herein presented.

O1 SB2

The authors acknowledge INNOCONCRETE project within Executive Programme on Scientific and Technological Cooperation between Italian Republic and the Kingdom of Sweden for the years 2018-2020, with the contribution of Ministry of Education of the University and Research of the Italian Republic.

Figure 1. Experimental setup used for the on-site measurements in Milan (Italy).

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O2 SB2

NOREPINEPHRINE AS NEW FUNCTIONAL MONOMER FOR MOLECULAR IMPRINTED OPTICAL BIOSENSORS: APPLICATIVE STUDY ON HUMAN AND CANINE BIOMARKERS

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Molecular imprinting gained increasing attention over the last two decades and today it represents a viable approach for the development of sensing platforms toward a wide panel of analytes, including biomarkers [1]. Over the years, various functional monomers have been exploited for the assembly of the polymeric network that embeds the selective cavities for the target. In this framework, dopamine has been widely investigated as monomer in non-covalent imprinting, however the uncontrollable surface morphology and the formation of precipitates during the polymerization may limit its applications. Like dopamine, norepinephrine (NE, or noradrenaline) is a neurotransmitter belonging to the family of catecholamines and it is able to easily self-polymerize in alkaline conditions, by forming adherent films on various type of surfaces, such as noble metals, metal oxide, glass and synthetic polymers [2].
In the last ten years polynorepinephrine (PNE) has been investigated in coating chemistry, while NE appears in few examples as template for molecular imprinting [3]. However, to the best of our knowledge, the only example of NE used as functional monomer for the development of a recognition element, consists in a solid phase polymer for the enantio-separation of some amino acids and other small molecules [4].
Two surface properties distinguish PNE from polydopamine (PDA): 1) the increased hydrophilicity due to the presence of the hydroxyl group in the benzylic position of NE; 2) the smoothness at the nanometer scale, given by the intermediate 3,4-dihydroxybenzaldehyde (DHBA) that reduces the surface roughness [5, 6]. In the context of molecular imprinting, the higher hydrophilic nature of PNE could reduce the non-specific adsorption of proteins thus favoring the selective binding of the target analyte.
Here we present the first example on the use of norepinephrine (NE) as functional monomer for imprinted optical biosensors, and possible application to molecular diagnostic i.e. the detection of human troponin I (TnI) and canine procalcitonin (PCT), crucial biomarkers for acute myocardial infarction (AMI) and sepsis, respectively. Moreover, we investigated the advantages of the epitope approach, in PNE imprinting, wherein only short peptides are printed, by overcoming the drawbacks of the whole protein imprinting (e.g. non-specific binging, instability, high cost). The imprinting of PNE has been performed on gold sensor chips and the efficiency of the relative optical biosensors has been investigated by SPR transduction. As result, PNE has proved to be a promising candidate for developing

biomimetic receptors by molecular imprinting with application to biosensing. It represents an interesting alternative to PDA, improving eventually the analytical sensor performances by reducing the non-specific binding of matrix components to the chip surface.

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O3 SB2

NANOCELLULOSE AND ELECTROCHEMICAL BIOSENSORS: A NEW TOOL TO REPAIR PAPER ARTWORKS

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In the last years from researchers and industry the cellulose nanocrystals (CNCs) have recently gained great attention because of their unique properties, including high tensile modulus, high specific surface area, biodegradability, biocompatibility and sustainability [1]. Moreover, they are rich in surface hydroxyl group and display a good affinity to a variety of materials, including conventional paper (that is made up mainly by cellulose fibers). Importantly, CNCs are nano-dimensioned rods with average length of few hundreds of nanometers and width corresponding to that of an elementary fibril (10 nm). These characteristics fit very well with water pools found in amorphous cellulose that is present in degraded and fragile paper artworks. Thus, CNCs can be applied to repair high quality paper with special surface properties or modify other solid surfaces [2,3]. In this context, we propose a new procedure to restore the paper materials by filling water pools using CNCs, which were prepared and functionalized ad hoc in laboratory as “chemically interacting filler”. CNCs are also transparent, and their deposition on the paper artwork will not compromise the precious contents that, by definition, a restoration process must preserve. The conservation strategy, proposed herein, is followed using a disposable non-invasive and compatible real time monitoring tool, based on an amperometric biosensor coupled with a sampling flow system/Gellan gel (used in the past to monitor the cleaning process of paper artworks [4]). In the present work, the amount of unsuccessfully adsorbed nanocellulose is measured by using an amperometric biosensor, where microbial cellulases (from Trichoderma sp. and/or Aspergilluse sp.) are immobilized. In order to assess the validity of this approach, several invasive and not invasive techniques, such as, fluorescence microscopy, SEM, FTIR-ATR, HPLC, XRD have been used [5].

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O4 SB2

MINIMALLY-INVASIVE MICRONEEDLE-BASED BIOSENSOR ARRAY FOR TRANSDERMAL SIMULTANEOUS LACTATE AND GLUCOSE MONITORING

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Microneedle arrays for minimally invasive continuous sensing in the dermal interstitial fluid (ISF) have been demonstrated in both amperometric [1,2] and potentiometric [3] modes, however there are no publication where microneedle arrays have been shown to function as second generation biosensors [4]. Here we report the first mediated pain free microneedle-based biosensor array for the continuous and simultaneous monitoring of lactate and glucose in artificial interstitial fluid (ISF).

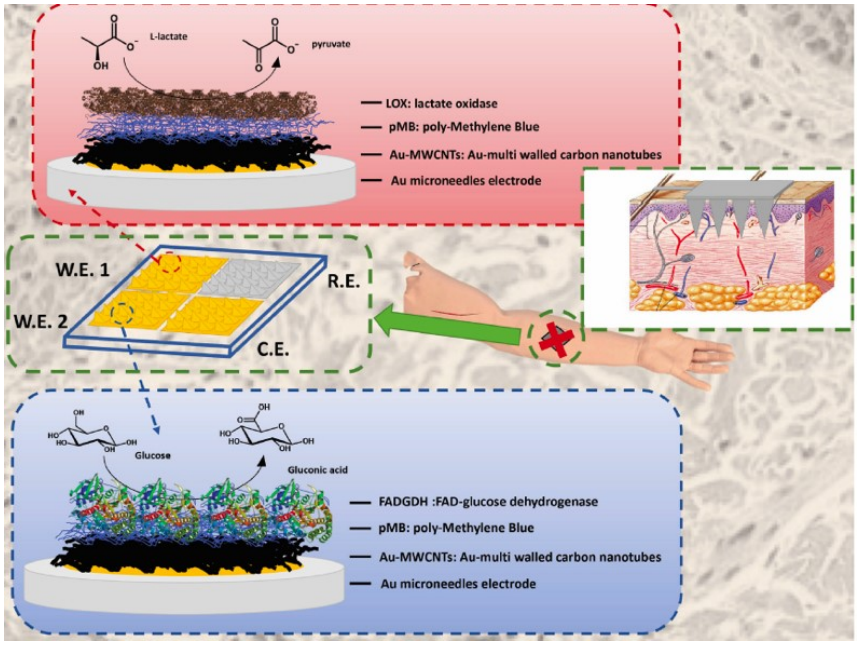

Figure 1. Schematic representation of microneedle-based biosensor array for simultaneous determination of glucose and lactate in ISF.

The gold surface of the microneedles has been modified by electrodeposition of Au-multiwalled carbon nanotubes (MWCNTs) and successively by electropolymerization of the redox mediator, methylene blue (MB). Functionalization of the Au-MWCNTs/polyMB

platform with the lactate oxidase (LOX) enzyme (working electrode 1) and with the FAD-Glucose dehydrogenase (FADGDH) enzyme (working electrode 2) enabled the continuous monitoring of lactate and glucose in the artificial ISF. The lactate biosensor exhibited a high sensitivity (797.4 ± 38.1 μA cm^{-2} mM^{-1}), a good linear range (10-100 μM) with a detection limit of 3 μM. The performances of the glucose biosensor were also good with a sensitivity of 405.2 ± 24.1 μA cm^{-2} mM^{-1}, a linear range between 0.05 and 5 mM and a detection limit of 7 μM. The biosensor array was tested to detect the amount of lactate generated after 100 minutes of cycling exercise (12 mM) and of glucose after a normal meal for a healthy patient (10 mM).

The results reveal that the new microneedles-based biosensor array holds interesting promise for the development of wearable real-time monitoring devices to be used in sport medicine and clinical care.

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O5 SB2

ELECTROANALYTICAL DETERMINATION OF CANNABIDIOL AND CANNABINOL IN AQUEOUS SOLUTION USING AMPEROMETRIC SENSORS

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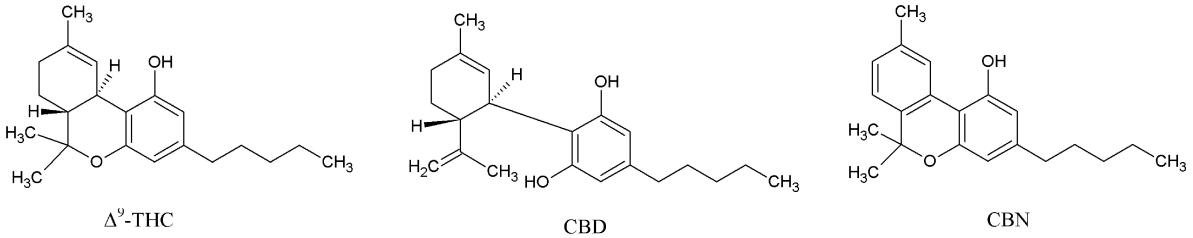
Cannabinoids are terpenophenolic compounds which have been extensively investigated due to their pharmacological properties. They can be classified in three main groups: endocannabinoids, phytocannabinoids and synthetic cannabinoids. Phytocannabinoids are derived from Cannabis Sativa L, widely known for the psychoactivity of the trans-Δ^9-tetrahydrocannabinol (Δ^9-THC, Scheme 1), one of the most active cannabinoids. By contrast, cannabidiol (CBD, Scheme 1), which is also found in cannabis, constitutes the major non-psychoactive cannabinoid of the plant. Several therapeutic properties can be attributed to this compound, such as anti-inflammatory, analgesic and neuroprotective, among others. Another type of phytocannabinoid is cannabinol (CBN, Scheme 1), a non-psychoactive product obtained from the degradation of the Δ^9-THC.



Scheme 1. Molecular structures of Δ^9-THC, CBD and CBN.

Cannabinoids are generally determined in real samples by chromatographic techniques, but the longtime and the non-portable instrumentation required for the analysis represents severe drawbacks. Electrochemical sensors have emerged in many frames as excellent alternatives to chromatographic techniques, thanks to the low cost of the instrumentation, the possibility to miniaturize the measuring device and the simplicity of use. Δ^9-THC, CBD and CBN are all electroactive, due to the irreversible oxidation of the phenol group; thus, a study concerning the electrochemical behaviour of these compounds constitutes an interesting topic of research in view of the development of an electrochemical method for their fast detection.

Several electrodic materials have been developed and tested in our lab for this specific electrochemical application. In the present study, we propose the use of a specific silicon

oxide-based graphitic material, the Sonogel-Carbon, containing a conducting polymer, the poly-(3,4-ethylenedioxythiophene) (PEDOT) [1]. The so called Sonogel-Carbon-PEDOT electrodes have been tested for the electrochemical determination of CBD and CBN from an aqueous/ethanol mixture, showing better performances with respect to other electrodic materials conventionally employed in electroanalysis, such as platinum and glassy-carbon.

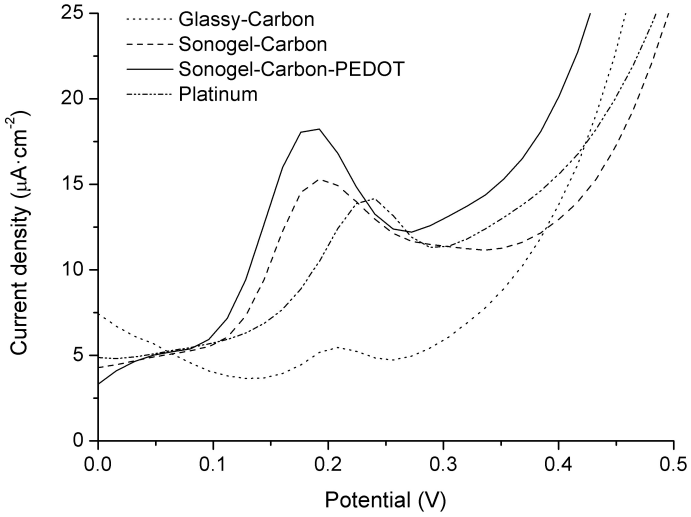


Figure 1. Differential Pulse Voltammetry responses recorded at different electrode materials in the same solution of CBD.

The analytical parameters obtained, in terms of sensitivity, limits of detection and quantitation, are comparable to those obtained for other electrochemical sensors. Furthermore, the possibility to easily renew the electrode surface by a simple and fast polishing method constitutes a valuable feature of the proposed sensor. Based on the experimental results, the electroanalytical method developed can represent a valuable tool for the rapid determination of these types of cannabinoids.

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O6 SB2

ELECTROCHEMICAL SENSOR FOR L-ARABITOL BASED ON 3D-GOLD NANOELECTRODE ENSEMBLES MODIFIED WITH MOLECULARLY IMPRINTED POLYMERS

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Triggered surface responsiveness paves the way for smart sensor technologies that not only have tunable recognition capability, but also provide sensing through a 'built-in' programming of the electrode material. There is also a steadily growing interest in smart recognition systems using synthetic analogs of antibodies, which offer improved stability, cost effectiveness and a means of rapid fabrication. Molecularly imprinting technology could provide a promising alternative and direct approach to determine a template used to create mimetic receptors by the formation of a specific polymer network.
A highly selective and sensitive sensor for L-arabitol is developed combining the advantages of three-dimensional nanostructrured electrodes, namely, 3D-ensembles of gold nanowires (3DNEE), with the recognition capability of molecularly imprinted polymer (MIP) [1]. L-arabitol is classified as one of the top 12 biomass-derivable building block chemicals, with low-calorific, low-glycemic, anticariogenic, and prebiotic character. In humans, abnormal concentrations of arabitol indicate the existence of infections by *Candida spp*. or other pathological conditions. The MIP/3DNEE is prepared by controlled etching of polycarbonate templated nanoelectrode ensembles, followed by electropolymerization of o-phenylenediamine on the gold nanowires in the presence of L-arabitol as a template molecule, followed by an ethanol/water mixture extraction. Electrochemical charactrization and analytically usefull signals are obtained using the ferrocenylmethytrimethylammonium cation as an electroactive probe which can access the MIP cavities, furnishing voltammetric signals which scale inversely with the L-arabitol concentration. The sensor is characterized by a low detection limit (7.5×10^{-10} mol L^{-1}) and can be applied also for quantifying L-arabitol concentration in real samples such as sugarcane vinasse.

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O1 FN1

UNVEILING THE COMPLEX KETO-ENOLIC TAUTOMERISM OF OLIVE OIL SECOIRIDOIDS THROUGH LIQUID-CHROMATOGRAPHY WITH FOURIER TRANSFORM MASS SPECTROMETRY INTEGRATED BY H/D EXCHANGE

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Prototropic tautomerism relating carbonylic compounds to the corresponding enolic counterparts has been the object of extensive fundamental research for decades. In particular, the role of enols and enolate ions as intermediates in several important organic or biochemical reactions has been emphasized [1]. Tautomerization constants and pKa values of hydrogen atoms involved in prototropism have also been evaluated through computational approaches [2]. Due to their generally low concentration [3], instability [4] and sensitivity to external factors, like solvent polarity [5], intermolecular interactions and temperature, the detection and quantitation of enolic tautomers, mainly faced by spectroscopic techniques like NMR, UV and IR, is usually a hard challenge, posing a high risk of inaccuracy [6]. Low temperature (-20 to -50°C) high performance liquid chromatography has thus been proposed as a less ambiguous approach to separate, and thus recognize distinctly, stable enolic tautomers, like those related to β-dicarbonylic compounds [7]. The use of mass spectrometry (MS) integrated by hydrogen/deuterium (H/D) exchange has subsequently been applied to recognize enolic tautomers from the mass increase occurring upon deuteration of enol-related labile hydrogen atoms [8]. Recently, the coupling of Reverse Phase Liquid Chromatography with ElectroSpray Ionization-Fourier transform MS (RPLC-ESI-FTMS), integrated by H/D exchange, has been adopted in our laboratory to unveil the presence of solution-stable tautomers for a compound belonging to the class of olive oil secoiridoids, oleuropein aglycone (OA) [9]. Extracted ion current chromatograms (XIC) referred to the major isotopologues of non-deuterated (A), mono-deuterated (B) and bis-deuterated OA anions of oleuropein aglycone, like those shown in Fig. 1, can be obtained after the RPLC-ESI(-)-FTMS analysis of an olive oil extract performed using D_2O as co-solvent of acetonitrile in the mobile phase. A careful examination of possible deuteration sites occurring on different OA isoforms led to recognize the elution of stable enolic/dienolic tautomers, having specific retention times and undergoing H/D exchange already in solution. In the present communication, the application of the same approach to three further relevant secoiridoids of olive oil, namely, ligstroside aglycone, oleacin and oleocanthal, all potentially able to generate enolic/dienolic tautomers, will be described in detail. In particular, the effect of mobile phase composition on the stabilization of secoiridoid enolic

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tautomers, favouring deuteration of labile enolic hydrogen atoms before ionization, will be discussed. In addition, the occurrence of H/D exchange also during the ionization process, especially in the charged microdroplets formed at the tip of the ESI needle, with interesting effects of competition for ionization between different sites of secoiridoids molecular structure and influence on MS/MS fragmentation patterns will be evaluated.

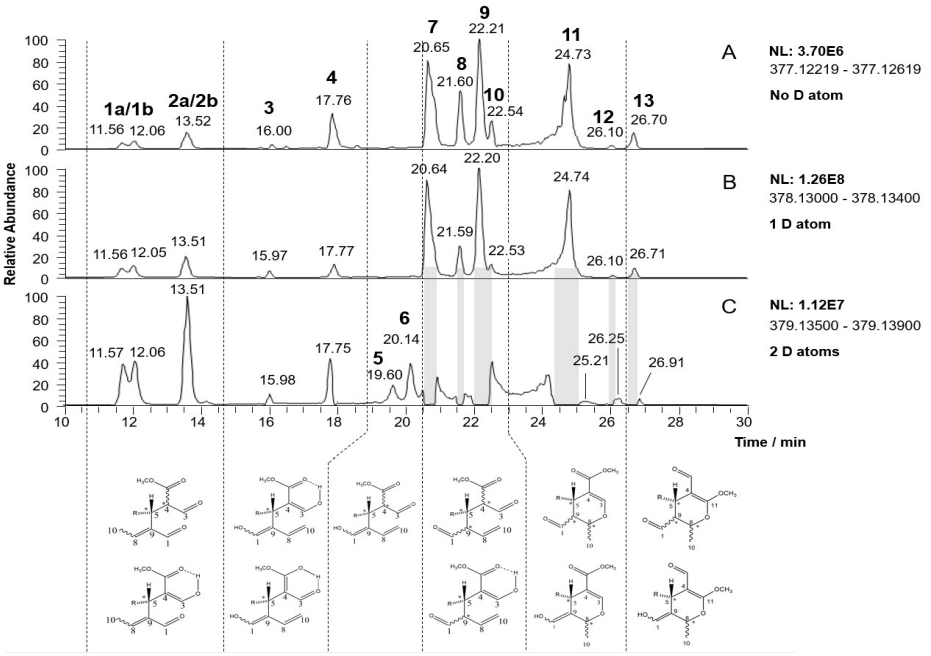

Figure 1. Comparison between eXtracted Ion Current (XIC) chromatograms related to non-, mono- and bis-deuterated isoforms of oleuropein aglycone (OA), obtained after the RPLC-ESI(-)-FTMS analysis of an olive oil extract under H/D exchange conditions, induced by using D_2O as co-solvent of acetonitrile in the mobile phase.

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O2 FN1

DETERMINATION OF THE PHENOLIC PROFILE IN BRASSICA JUNCEA OF DIFFERENT SPECIES BY COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

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Brassica vegetables are known to contain a high concentration of bioactive compounds such as hydroxycinnamic acids and flavonoid derivatives, which play an important role in the prevention of several pathologies e.g. coronary heart diseases and cancer, especially of the gastrointestinal tract. In particular, the molecules responsible for the beneficial effects are polyphenols and glucosinolates.
For their characterization, different studies have been carried out by conventional one-dimensional liquid chromatography even though it can present some limits especially in terms of resolving power.
A powerful alternative is represented by comprehensive two-dimensional liquid chromatography (LC×LC), where two columns of different selectivity are separated by means of two switching valves. The aim of this study was to investigate three different cultivars by using reversed phase conditions in both dimensions and specifically cyano column (250 × 1 mm I.D, 5 μm dp) and a RP-Amide column (50 x 4.6 mm, 2.7 μm dp) in the first (^{1}D) and second (^{2}D) dimensions, respectively. Moreover, to improve the separation efficiency in the ^{2}D a segmented in-fraction gradient was employed, this allowing a further increase of the overall theoretical, effective and corrected peak capacity of the LC×LC system.
Interestingly, one of the most recent achievements is the possibility to employ a micro LC pump in the first dimension of the LC×LC, allowing high reproducibility and stable retention times was also evaluated.
The samples, represented by dried and powdered leaves, chosen for their complexity, were extracted by liquid-liquid extraction procedure using MeOH/Water (60:40 v/v). The recovery yield was calculated by adding apigenin as internal standard at the beginning of the extraction procedure.

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Forty-five compounds were separated and identified through PDA and MS (ESI^-) detection, in three different cultivar of Brassica juncea.
Quantification was carried out through three different standards (quercetin-3-O-glucopyranoside, isorhamnetin-3-O-glucoside and kaempferol-3-O-glucoside), the calibration curves were created automatically by the use of Cromsquare software and the method was validated yielding satisfactory LODs and LOQs values.
The comprehensive approach demonstrated its validity in the analysis of complex matrices, such as Brassica extracts. Furthermore, the characterization of these samples will aid to confirm their potential use for the human health.

The authors thank Shimadzu and Merck KGaA for the continuous support.
This work has been carried out within the framework of the Research Project PRIN 2015: Securing and ensuring sustainable use of agriculture waste, co- and by-products: an integrated analytical approach combining mass spectrometry with health effect-based biosensing, supported by the Italian Ministry of University and Scientific Research, no. 2015FFY97L.

O3 FN1

UNRAVELLING THE BIOACTIVITY POTENTIAL OF COMPLEX MATRICES: FOCUSING ON LIPIDS AND UNUSUAL AMINO ACIDS IN OILS

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The recent interest in the characterization of naturally occurring compounds is mainly driven by their potential health promoting bioactivities. Metabolites found in food and vegetables represent some of the mainly investigated analytes for the discovery of new bioactive compounds, which can later be exploited to valorize commercial products or find valuable natural sources of nutraceutical compounds.
Within this framework, extra virgin olive oil represents a typical product of the Mediterranean area since antiquity, known and appreciated also outside the boundaries of the Mediterranean Sea. Apart from the well-known content in polyphenols, several other metabolites characterize extra virgin olive oil and contribute to the bioactivity [1]. Among such compounds are polar lipids and trace metabolites, such as seleno-amino acids. As far as the polar lipids are concerned, extra virgin olive oil has a low content of phospholipids, compared to other vegetal oils, but they potentially provide interesting bioactivities, also in relation to different diseases and symptoms, such as inflammation, cholesterol absorption, coronary heart diseases, and cancer [2]. Moreover, these compounds could be used for authentication studies, being the phospholipid profile of olive oil peculiar [3]. Due to their low abundance, an enrichment method was devised to improve the phospholipid coverage, based on solid phase extraction on graphitized carbon black, liquid chromatography coupled to high resolution tandem mass spectrometry and bioinformatic analysis by Lipostar. A method was validated for target lipid standards, then applied to characterize by untargeted analysis extra virgin olive oils from different regions. As far as seleno-amino acids was concerned, they represent an important form of organic selenium, an essential micronutrient for humans [4]. Two methods were developed for direct enrichment of seleno-amino acids in oils. In the first method, a Chirobiotic TAG precolumn was employed to preconcentrate the analytes under Normal Phase (NP) conditions. Oil samples were diluted with dichloromethane and loaded on the precolumn using nitrogen as pressurizing gas. The use of NP allowed to efficiently eliminate oil traces and trap the analytes with high recovery. In the second method, a different sample preparation strategy was pursued, based on liquid-liquid seleno-amino acids extraction and -up by reversed phase/strong cation exchange OASIS MCX solid phase extraction. The second procedure allowed to lower both method detection and quantification limits below 1 ng g^{-1}. Both enrichment methods were coupled with an enantioselective separation of the target compounds (selenomethionine, selenocystine and selenocysteine) and triple-quadrupole single-reaction-monitoring mass-

spectrometry acquisition. Both methods were validated and finally applied to commercial oil samples and Italian extra virgin olive oils.

This work has been supported by AGER 2 Project, grant n. 2016-0169, "Valorizzazione dei prodotti italiani derivanti dall'oliva attraverso tecniche analitiche innovative (VIOLIN)".

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O1 MAT

SYNTHESIS OF Co BASED LAYERED DOUBLE HYDROXIDES: TOWARD A NOBLE METAL FREE ELECTRO-CATALYSIS AND SENSING

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An electrosynthesis protocol that allows the deposition of thin films of Co/Al and Co/Fe layered double hydroxides (LDHs) on two different supports (Platinum and Grafoil) is hereby described that is more efficient than the one previously proposed by our group. The first material was chosen as a reference for the synthesis, since we had already investigated the electrodeposition mechanism on Pt. Grafoil, instead, was chosen since it can be considered a more versatile substrate to be used in a wide range of applications: it is carbon based, low cost, flexible and environmentally friendly.
The synthetic approach is based on potentiodynamic cathodic reduction of nitrates which is a complex process which leads to a pH increase next to the electrode, necessary for the precipitation of the double hydroxide. [1,2] All the films have been characterized by a lot of analytical techniques including cyclic voltammetry (CV), powder X-ray diffraction (PXRD), scanning electron microscopy (SEM), couple with EDS analysis, Raman and atomic emission spectroscopies. Moreover, LDHs electro-synthesized on Grafoil have also been investigated by X-ray absorption spectroscopy (XAS) to better understand their chemistry and their local metal structure (Fig. 1).
All characterizations confirm the LDH structure of the electro-synthesized materials. In particular, XRD suggests better-formed crystal domains with respect to the previously proposed electrochemical approach, based on a potentiostatic method. [3] Moreover, another remarkable result is related to the morphology of the Co-containing LDHs on Pt, which displayed a tubular nanostructure for Co/Fe-LDs and a cauliflower 3D hierarchical morphology for Co/Al-LDH.
Finally, the modified electrodes were employed both for the sensing and the electro-oxidation of 5-(hydroxymethyl)furfural (HMF), displaying promising performances.
Nowadays, the HMF molecule is considered a fundamental platform chemical, i.e. it is a key precursor for a great number of compounds which find application in the fuel and polymer industry. Among several transforming options of HMF, more and more research focus on the oxidation of HMF to 2,5-furandicarboxylic acid (FDCA) as FDCA may replace terephthalic acid in order to produce environmentally friendly plastic materials, as an alternative to polyethylene terephthalate (PET). Therefore, the detection of this compound, and the investigation of stable catalysts able to selectively oxidize HMF to FDCA and with high yield are nowadays relevant topics and a step forward for a green world and economy.

O1 MAT

All the modified electrodes were tested as sensors for HMF using chronoamperometry at +0.5 V vs SCE, after a preliminary investigation by CV in order to establish the suitable voltage conditions. The sensitivity and the limit of detection values were found to be 0.417 A/(Mcm^2) and 1.69 x 10^{-4} M for the best performing system, respectively.
As to the study of HMF oxidation, an exhaustive electrolysis was carried out, and HPLC, ^{1}H NMR and ESI-MS were utilized to identify and quantitate the reaction products. Two principal products were detected: 5-Hydroxymethyl-2-furancarboxylic acid (HMFCA) and FDCA, but also an unidentified compound was observed. The HMF conversion resulted of 100%.
In conclusion, we propose a robust and highly reproducible electrosynthesis procedure that can be applied to different conductive supports and can be employed in several fields of interest, such as sensing and industrial electrocatalysis.

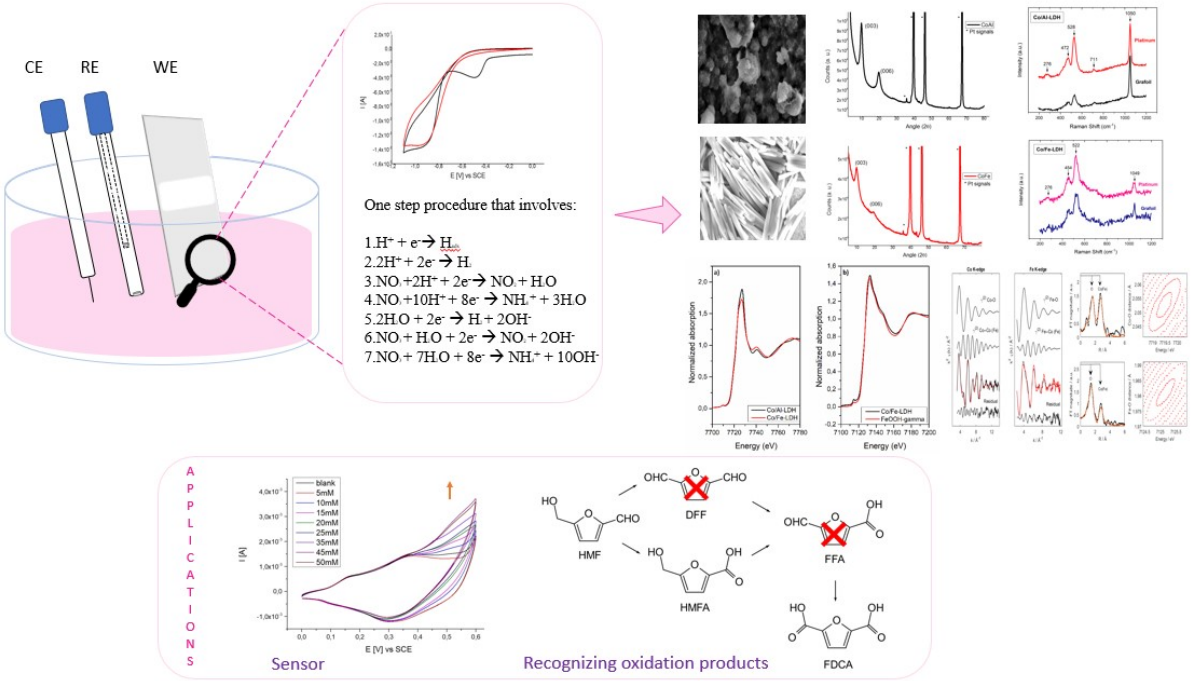

Figure 1. Scheme of the proposed work.

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O2 MAT

GRAPHENE DERIVATIVES: SYNTHESIS, CHARACTERIZATION AND APPLICATION IN GAS SENSING DEVICES. RAMAN STUDY OF THE INTERACTIONS AMONG NO, NO_2 GASEOUS POLLUTANTS, AND THE GRAPHENE GAS SENSITIVE LAYERS

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In this work, the chemical synthesis of graphene derivatives have been performed with two different graphite, applied as graphene precursors, by using the modified Hummer's method [1]. Raman, FTIR and TEM characterization were carried out to identify the presence of high quality graphene materials. The highest quality of graphene sample, obtained working with the micro-metric graphite from Merck (as solid powder precursor), was applied for the subsequent functionalization with Triethanolamine (TEOA). FTIR study has been also performed to verify the functionalization of graphene nanosheets with TEOA. This aliphatic amine has been selected because it results highly sensitive [2] and selective toward the NO_2 uptake [3], present as primary pollutant in troposphere. Theoretical studies (based on Computational models) [4], reveal that the unmodified graphene (i.e. pristine graphene, as deposited) is not an ideal material for gas sensing and for this purpose graphene needs to be functionalized. Especially, FTIR reveals the presence of OH groups on graphene nano sheets and the First-principles calculations also demonstrate the main role of the hydroxyl groups [5] in NO_2 sensing (during the uptake of the gaseous pollutants and their release, especially when sensors are regenerated). Moreover, Raman spectroscopy clearly highlights the reversible molecular interactions between graphene derivative and NO_2 gaseous pollutant, in both cases: during the selective uptake/capturing and also during the gaseous pollutants desorbing step (this latter necessary for the regeneration of the sensor nanoplatform). Finally, derivatization of Graphene is the first step in designing air pollution sensors characterized by sufficiently moderate cost and ease of operation. These would be very suitable for "saturation monitoring" in which many sensors per unit surface may contribute to a better knowledge of the spatial distribution of atmospheric pollution and a better evaluation of its effects on population.

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O3 MAT

SPECTROSCOPY AND MICROSCOPY EVIDENCES OF CANNIBALISM EVENTS IN THE *IN-SITU* ASSESSMENT OF LONG-TERM BIOFILM-ANTIMICROBIAL INTERACTIONS: A NEW VIEWPOINT ON ANTIMICROBIAL RESISTANCE?

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Differently from planktonic state, bacterial biofilms can be considered the major cause of serious health issues in human medicine and food industry, due to their ensuing resistance against harsh conditions and pharmacological treatments [1]. Biofilms are defined as three-dimensional structures comprising cells rooted in a matrix build up by extracellular polymeric substances (EPS) [2]. This intricate system is dynamic, and its structure is strongly influenced by a plethora of parameters such as biofilm oldness and exterior conditions, like nutrients deficiency, and/or attack of various agents [3]. Biofilm formation is a chemically complex multi-stage process, in which bacteria transmute from planktonic form to sessile mode; the interaction that occurs between bacteria in a biofilm and its surrounding environment can largely determine the extent and the composition of the bacterial colonies. Moreover, bacterial colonies can activate survival strategies when subjected to stress conditions (i.e. presence of antimicrobial agents). Occasionally, cannibalistic behavior may occur [4], which involves the secretion of cannibalism toxins, which can kill sensitive bacteria of the same colony. Thus, generated lysed cells may then provide nutrients for the cannibals. Several new methodologies have been recently developed for or adapted to biofilm formation studies aiming at a comprehensive understanding of biofilm physiology, structure and composition, to find novel and more effective eradication strategies. Among them, Fourier transform infrared spectroscopy (FTIR) –especially in attenuated total reflectance (IR-ATR) mode – may provide *in-situ* and real time monitoring of biofilm lifecycles with molecularly specific details on the first attachment stages. Biofilm growth may occur during extended timespans; therefore, not only long-term effective bacterial treatments are required, but also appropriate analytical methods, to study the long-term behavior of biofilms. Due to the well-known biofilm antibiotic resistance [5], it is nowadays of increasing interest to develop innovative methodologies for the treatment of biofilm-related infections. In our laboratories, these new strategies mainly exploit inorganic nanoparticles (NPs) with antimicrobial properties, such as ZnONPs, AgNPs, CuNPs, etc. [6]. In this work, AgNPs have been embedded in various polymeric matrices (fluoropolymer, polyethylene oxide, polylactic acid, etc.), which allowed for the preparation of organic thin films with tunable loading of inorganic (i.e. antimicrobial) NPs. First, composite morphology was investigated by electron

microscopies and atomic force microscopy (AFM); then, x-ray photoelectron spectroscopy was employed to gather information about surface chemical composition. Kinetics of antimicrobial ion release were also investigated, and nanocomposite behavior was correlated for the first time with its swelling properties and 3D modification after immersion in liquid medium for a long-time span. The investigation of biofilm growth and inhibition by the antimicrobial material has involved both imaging (AFM and optical microscopies), and spectroscopic (IR-ATR analysis) techniques. In particular, the infrared spectroscopic analysis of the biofilm allowed gathering molecular information on the biofilm development and behavior during long-term contact with an antimicrobial surface. A detailed comparison between the IR data obtained at differently modified ZnSe crystal surfaces allowed for a decoupling of the effect of antimicrobial ionic release from the thin film, from the direct contact between bacteria and antimicrobial thin film. We demonstrated that Ag^+ ions, released in the surrounding solution, exert a biocide action: ionic release can slow down surface colonization and eradicate the bacterial biofilm within few hours. We also demonstrated that bacterial cells can re-colonize on dead biomass, when the latter is thick enough to prevent a direct interaction with the antimicrobial surface.

In summary, this study represents an excellent foundation for investigating the contact between nanoantimicrobials and nascent biofilms over extended periods of time. Moreover, it paves the way to further studies on the long-term exposure of biofilms towards antimicrobial surfaces, and it could be useful also for a better understanding of the early stages leading to antimicrobial resistance phenomena [7].

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O1 SS3

RAPID SOLID LIQUID DYNAMIC EXTRACTION IS A FORERUNNER TECHNIQUE IN GREEN CHEMISTRY FOR THE SOLID-LIQUID EXTRACTION SECTOR. TWENTY YEARS REPORT

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In the past, liquid/solid extractions were performed with systems that required the use of different solvents, such as the Soxhlet method. Currently, everything has changed with the introduction of tools that reduce extraction times, reduce solvent consumption, improve extraction efficiency and reduce environmental impact (green extractions). All this is based on a new way of thinking about chemistry, the so-called green chemistry, based on the prevention of pollution that involves the development of systems that use and produce substances that are of lower risk for human health and low environmental impact. Exploiting the principles of green chemistry means implementing a series of principles that reduce or eliminate the use and production of hazardous substances during all stages of processing. In this context, the Rapid Dynamic Liquid Solid Extraction (RSLDE), through the use of the Naviglio extractor, represents a forerunner technique in the field of green extractions. The extraction takes place by generating a negative pressure gradient from the inside towards the outside of the solid matrix, so it can be conducted at room temperature or even sub-environment (Naviglio, 2003). Literature data have shown the versatility of RSLDE in various fields of application: pharmaceutical, cosmetic, herbalist, food and beverage and not least of food waste. Furthermore, the reproducibility of the extraction on the same matrix in weight terms was tested and comparison experiments were conducted with the other extraction techniques, which showed a higher recovery in favour of the RSLDE, as well as a higher extract quality and in no case it was detected alteration of thermolabile substances.

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O2 SS3

CHARACTERIZATION, SIZE DISTRIBUTION AND TIME-EVOLUTION ANALYSIS OF ENDOGENOUS NANOPARTICLES IN ITALIAN RED WINES

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The presence of colloidal particles in wine impacts properties, taste and palatability, and can vary greatly among wines of the same cultivar.
Wine macromolecules consist of proteins, polysaccharides and polyphenols [1]. The first two interact with polyphenols participating in aggregation, and originating nanoparticles which may have different composition [2]. This aggregation directly influences wine quality, its sensory characteristics such as the perception of astringency, and colloidal stability.
If the chemical composition of wine is widely studied, and analysis of the molecular and macromolecular content are routinely performed in the course of wine production, there are many gaps in the characterization of colloidal systems endogenously formed in wine.
One of the key steps of such a characterization is the ability to work in analytical conditions as close as possible to the colloid environment, to preserve the sample structure and avoid degradation. As a soft and versatile fractionation technique, asymmetrical flow field-flow fractionation (AF4) can provide native separation of wine colloidal matter while working in simulated wine as mobile phase. In our work, in the context of mapping wine properties and nano-structure, we analyzed a pool of Italian red wines by using an AF4-multidetector platform. The two directions of this study involved identifying the differences between different wines from the same cultivar, and the changes in the size distribution of the colloids over time. Different red wines were analyzed and characterized using UV, Fluorescence and Multi-Angle Light scattering (MALS).

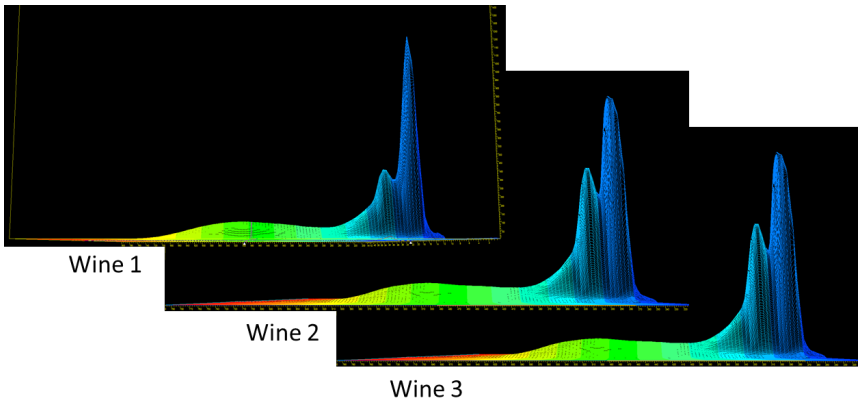

Figure 1. Absorption profile of nanosystems from different red wines

O2 SS3

From UV detection we collected online, 3D spectra showing the compresence of absorption maxima within the same peak, suggesting that proteins, polysaccharides and polyphenols might agglomerate together and possibly layer into nano-sized specimens. Fluorescence confirmed the presence of proteins in these systems, and showed to be useful in estimating the protein content via calibration with bovine serum albumine. MALS allowed the identification of the size distribution of these species, which ranged from 25 to 70 nm in terms of gyration radius (r_g). The difference between samples mainly consisted in the relative intensity of the absorption maxima, meaning that different nanoparticles can form from different protein-polysaccharide-polyphenol ratios. Moreover, the nanoparticles size varied between samples. Each wine was analyzed over time: the changes in r_g suggest the insurgence of conformational changes of the nanoparticles after the sample is exposed to air, implying a correlation between these systems and the organoleptic modifications which incur over aging.
Lastly, fractions of each wine were collected to facilitate downstream analyses, such as Mass Spectrometry.

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O3 SS3

OPTIMIZATION OF A RAPID AND GREEN ANALYTICAL METHOD FOR THE DETERMINATION OF PERFLUOROALKYL ACIDS IN FRUITS IRRIGATED WITH RECLAIMED WASTEWATER

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Agriculture is typically characterized by a high-water demand, since about 70% of worldwide freshwater (FW) withdrawals is used for agricultural irrigation [1]. On the other hand, limited FW availability is a problem of increasing concern and the reuse of treated wastewater (TWW) for irrigation could be an efficient tool of reducing water shortage. However, the TWW reuse is currently far to be fully realized, due to several barriers, such as potential risks for the environment and the human health in the reuse of wastewater improperly treated, due to residual concentrations of priority and/or emerging organic micropollutants (e.g. perfluorinated compounds) [2]. In this regard, fruits characterized by different chemical composition and water percentage may represent interesting models for this kind of investigations. Accordingly, strawberry and olive have been selected.
Based on the aforementioned considerations, this work focused on the development of an analytical method for the identification and determination of selected linear perfluoroalkyl acids (PFAAs) in strawberries and olives fruits obtained by irrigation with TWWs. The method is based on the QuEChERS approach, which include the liquid/liquid partition of analytes between salty water and acetonitrile [3], combined with dispersive solid phase extraction (d-SPE) as clean-up step, followed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) analysis. Different d-SPE sorbent phase – i.e. octdecylsilica (C18), primary secondary amine (PSA) and graphitized carbon black (GCB) – have been tested in order to evaluate the best compromise between matrix effect (ME%) and analyte recoveries. For strawberry, the d-SPE clean-up step was found unnecessary, since even in absence of the extract purification, signal suppressions or enhancements lower than 20% were observed with the sole exception of perfluorohexanesulphonic acid (PFHxS), which showed a signal suppression of about 35%. Recoveries included in the range of 83-111% were achieved. Method quantification limits were in between 3.9 and 477 *pg* g^{-1} d.w. Hence, the method herein proposed represented a general improvement in terms of simplicity and total analysis time, as well as sensitivity in comparison with previously published methods focusing on the determination of PFAAs in strawberry and other small fruits [4-8].
Conversely, for olives the use of the d-SPE clean-up step was mandatory, due to the high amount of co-extracted fatty matrix components that influence the chromatographic behavior of target analytes, as well as overall performances of the analytical method. Among

the d-SPE phases investigated, C18 and GCB provided the best results, whereas PSA gave rise to retention of target analytes due to its character of anion exchanger. The d-SPE clean-up allowed for obtaining extracts that can be analyzed with reproducible results and without problems of method robustness. However, the absolute value of matrix effect remained high, thus making necessary the matrix matched calibration approach and/or the use of spiking procedure with labelled PFAAs for the analysis of real samples. More in detail, a suppressive ME% was observed for the investigated PFAAs with the only exceptions of perfluorobutanesulphonic acid (PFBuS) and perfluoropentanoic acid (PFPeA), which were amplified. High recovery (i.e. > 75%) and sensitivity (tens-hundreds of $pg\ g^{-1}$ d.w.) have been obtained also for olives, indicating that the QuEChERS procedure is suitable for the analysis of PFAAs in fruits characterized by very different chemical composition.

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O1 FN2

POSSIBLE EFFECTS OF CULTIVAR, GEOGRAPHICAL ORIGIN AND TECHNOLOGY ON THE SECOIRIDOID CONTENT OF ITALIAN EXTRA-VIRGIN OLIVE OILS (EVOOs): AN INVESTIGATION BASED ON LIQUID CHROMATOGRAPHY WITH ELECTROSPRAY IONIZATION AND FOURIER TRANSFORM MASS SPECTROMETRY

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Secoiridoids represent the main class of phenolic compounds found in olive (*O. europaea*) leaves and drupes and have raised a significant research interest in recent years due to their antioxidant, anti-inflammatory and anti-carcinogenic effects [1]. Four main secoiridoids are transferred into the olive oil upon drupe crushing, namely, oleuropein and ligstroside aglycones, resulting from the hydrolysis of the corresponding glycosides (found in olive drupes and leaves) catalyzed by a β-glucosidase, and oleacin and oleocanthal, generated upon aglycone decarboxymethylation catalyzed by the synergic action of a methylesterase and a decarboxylase [2]. Therefore, the secoiridoid content of extra-virgin olive oil (EVOO) is generated by a wide number of factors and processes, such as olive cultivar, ripening stage and geographical origin, agronomic practices and oil production technology. Due to genetic factors that regulate the expression of phenolic compounds, some olive cultivars are richer in secoiridoids than others (even if the ripening stage is the same) and these differences are reproduced in olive oils. Furthermore, the same cultivar grown in different regions of the same country may show differences in phenolic composition related to biotic and abiotic effects (i.e., soil characteristics, precipitation, temperature, humidity). In addition, technology and especially the type of horizontal centrifugation used for olive oil extraction, affects secoiridoids quantity and, consequently, the nutritional and sensory properties of the final product [2].
A higher content of phenolic compounds, including secoiridoids, has been often reported for EVOO obtained by two-phase centrifugation (i.e. not implying water addition), compared to three-phase centrifugation, even if only a few studies have been focused on the effect of centrifugation on the secoiridoid contents [2]. In the context of a national research project on olive oil (VIOLIN, *Valorization of Italian OLive products through INnovative analytical tools*) an investigation of the main sources of variability affecting the secoiridoids abundance in olive oils has been recently undertaken in our laboratory, involving a relevant number of

samples from different Italian regions, differing also for the horizontal centrifugation approach.

Secoiridoids were extracted in duplicate from EVOO samples using a CH_3OH/H_2O 60:40 (v/v) mixture, following the protocol reported by Vichi *et al.* [3], with some modifications. Each extract was then analyzed by RPLC-ESI-FTMS, using a hybrid quadrupole-Orbitrap mass spectrometer. Quantitative data relevant to the four main secoiridoids, expressed as chromatographic peak areas normalized to that of oleuropein, added to oil extracts as internal standard, were subsequently subjected to chemometrics, namely, Cluster Analysis (CA) and Principal Component Analysis (PCA). As an example, the bidimensional scatterplot obtained for the first two principal components in the case of olive oils belonging to the 2016-2017 campaign is reported in Figure 1. As apparent, oil samples were generally separated in terms of the adopted horizontal centrifugation, with some exceptions, that could be explained considering other features, like cultivar or region. Further examples of the chemometrics-based evaluation of the effects of production features on the secoiridoid content of olive oil will be provided during the present communication.

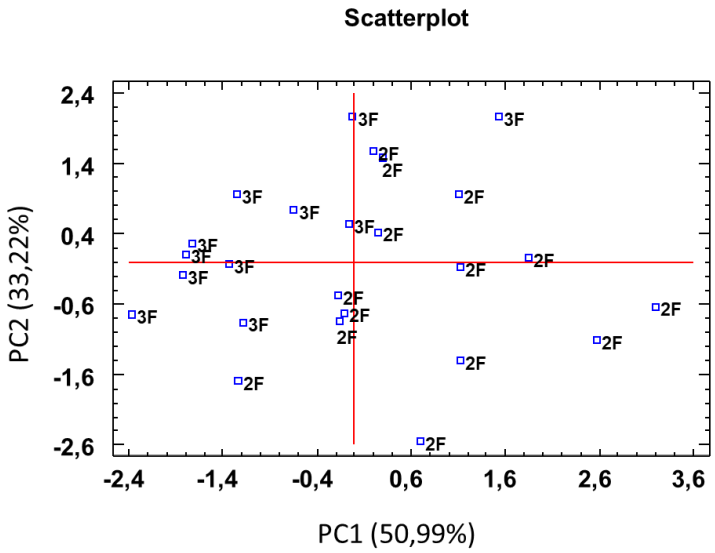

Figure 1. Scatterplot referred to the first two components (PC1 and PC2) obtained after PCA on normalized XIC peak areas of oleuropein aglycone, ligstroside aglycone, oleacin and oleocanthal, resulting from the LC-ESI-FTMS analysis of 25 Italian extra-virgin olive oils produced using three- (3F) or two-phase (2F) decanters.

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O2 FN2

ANTIOXIDANTS AND NEW PRODUCTS FROM *OLEA EUROPAEA L.*

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Products and by-products from *Olea europaea* L. are promising sources of bioactive compounds. Particularly, tyrosol (Tyr), hydroxytyrosol (HTyr) and oleuropein (Ole) are among the major phenolic compounds present in fruits and leaves, and offer several beneficial effects on human health [1].
Qualitative and quantitative analyses of nutraceutical components contained in olive fruits, olive oil (primary product), olive pomace (by-product from agro-industrial processing) and leaves (by-product from agricultural practices) were carried out.
The sampling of pomaces (as well as fruits and olive oils) was performed at harvesting/production season, whereas the sampling of leaves was performed at pruning time for five different cultivars (Leccino, Correggiolo, Frantoio, Dolce Agogia, Moraiolo). In the case of Leccino cultivar the collection was repeated seasonally, considering the months in which typical phenological changes in the olive tree life cycle occur. All the samples, after due pre-treatments, underwent extraction procedures, and were analyzed via spectrophotometric assays for total polyphenols content and antioxidant and radical scavenging activities. Total polyphenols content and antioxidant activity were analyzed in all samples (humid pomaces 2015: TPP, 26.0±1.5–43.7±3.0 g(GAEq)/kg dry weight, dw; TEAC/ABTS, 189.5±3.7–388.1±12.0 mmol(Trx)kg dw). Radical (DPPH) quenching potential was analyzed via photometric and EPR methods, obtaining Vis/EPR signals ratio by 1.05±0.45 and 1.66±0.39 for fruits and pomaces, respectively. Through HPLC-UV and HPLC-MS/MS techniques, the secoiridoid oleuropein and its derivative hydroxytyrosol, as well as selected hydroxycinnamic acids and flavonoids (like caffeic, ferulic and chlorogenic acids; and rutin and luteolin) were identified and quantified [2] (Figure 1).
It is well known, that most of polyphenol compounds are characterized by low bioavailability due to their low solubility in biological fluids, possible carriers have been considered. Liposomes are drug/nutraceutical delivery carriers, widely used for driving bio-active molecules to desired target tissues, protecting the encapsulated molecule from enzymatic metabolic processes and decreasing potential side effects [3,4].
In this study, zwitterionic liposomes containing tyrosol (Tyr), hydroxytyrosol (HTyr) and oleuropein (Ole) were synthesized and characterized for their size and surface charge. Particular attention was devoted to the determination of encapsulation efficiency (EE%), quantifying the loaded Tyr, HTyr and Ole amount, by using three different techniques: direct

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UV spectrophotometry, High Performance Liquid Chromatography (HPLC-UV) and Trolox Equivalent Antioxidant Capacity (TEAC) assay. The results revealed higher EE% for oleuropein (Figure 2). Cytotoxicity assay of liposomal preparations was also performed, against human chondrocytes showing any cytotoxicity at the tested concentrations.

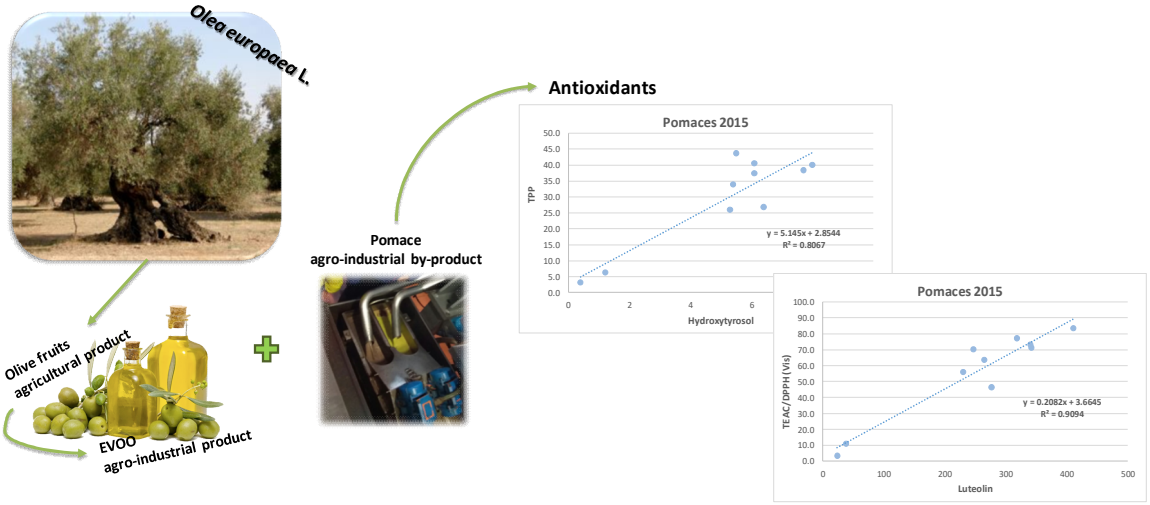

Figure 1. Chemical characterization and antioxidant properties of olive pomace, a by-product from olive oil production.

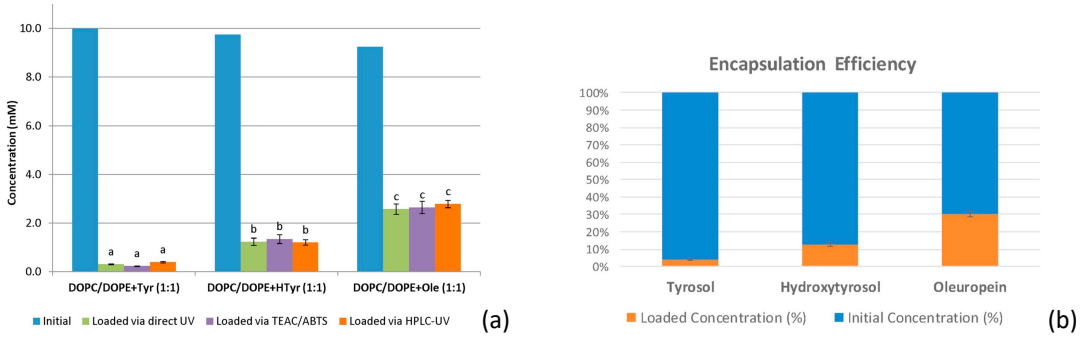

Figure 2. Graphical representation of the encapsulation efficiency (EE%) for the liposomes loaded with Tyr, HTyr and Ole, as determined via direct UV, TEAC and HPLC-UV analysis.

Tuscan Regional Administration is acknowledged for funding the project NUTRIforOIL. The project factory partners are also kindly acknowledged.

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O3 FN2

A RAPID MALDI MS/MS BASED METHOD FOR ASSESSING SAFFRON (*Crocus sativus L.*) ADULTERATION

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Saffron, the red dried stigmas of *Crocus sativus* L., is the world's most expensive spice and thereby is considered within the major candidates for economically motivated fraud. Saffron authentication through established methodologies is a challenging task, as saffron of higher quality may intentionally be blended with plant-derived adulterants. The most frequently used adulterants are saffron stamens, safflower, calendula, turmeric rhizomes or dried gardenia fruits [1,2]. Fruits of *Gardenia jasminoides* Ellis represent a bio-adulterant which is difficult to detect by classical methods, because it contains crocins (C-1÷C-3) and flavonoids as does saffron itself. The quality of saffron and its commercial value are determined by specifications described within the ISO/TS-3632 standard that established spectrophotometric (for picrocrocin and safranal) and chromatographic (for crocins and polar dyes) measurements. According to the ISO/TS-3632 standard, the maximum mass fraction of foreign matter permitted in the third-class products is 1% (w/w). The standard UV–vis spectrophotometric method of ISO 3632-2 for grading saffron may not reveal saffron adulteration with amounts lower to 20% (w/w) of safflower, turmeric, or calendula [3]. Many analytical methods have been developed for authentication of saffron, including strategies based on the use of NMR and LC-MS [4]. Mass spectrometry is a powerful tool for the high-throughput detection and quantitation of metabolites, several studies have shown that MALDI (Matrix-assisted laser desorption ionization) can be used as an alternative to LC-ESI for the highly sensitive analysis of low molecular weight compounds in complex matrices [5]. This MS technique is extremely advantageous due to short analysis times, high sensitivity, tolerance to contaminants, and the ability to detect different components in highly complex mixtures. MALDI MS/MS provided quantitation of target compounds and small sets of analytes in a complex matrix with great sensitivity, dynamic range, and precision. The common workflow requires the construction of a calibration curve with standard solutions containing the same (fixed) amount of the stable isotope internal standard, and variable amounts of the single specific analyte of interest. This approach is suitable only when stable isotope internal standards are available for each analyte of interest, and the analyte concentration levels to be measured are known. Synthetic isotope labeled markers of saffron are not available, therefore, to overcome this drawback, we evaluated the use of whole extracts obtained from sets of standard sample (w/w), with the addition of a non-isotopic isobaric internal standard (IIS). The method reported is a sensitive and fast quantitative MALDI-MS/MS method used to assess saffron authenticity by direct

analysis through the determination of picrocrocin as the saffron authenticity marker, and using curcumin as the non-isotopic isobaric internal standard. The internal standard curcumin yielded good linearity (R^2 = 0.994), and with confidence intervals at 95% for intercept. The detectable maximum adulteration percentage (99.0%) was estimated interpolating the limit of detection (LOD) for the isobaric internal standard in linear regression. The LOD was 47.63 ppm, and LOQ was 56.53 ppm. The capability of the MS approach to monitor analytes in a specific, selective fashion was used to obtain a semi-quantitative adulteration percentage and to establish the adulterant by additional experiments. Finally, the detection of gardecin and its derivatives in commercial samples indicated that *Gardenia jasminoides* Ellis was used as adulterant.

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O4 FN2

RETRIEVAL AND CHARACTERISATION OF BIOACTIVE PEPTIDES FROM TUNA PROCESSING WASTE BY MEANS OF UHPLC-MS

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Processing of raw fish into food products generates large quantities of by-products (meat, scales, head, viscera and roes), which are usually discarded and need to be disposed. However, many of them may exert nutritional value, related to the content in essential molecules to the human diet; thus, their re-use after processing of raw foodstuffs may surely improve the economic aspect of food processing industry and moreover positively impact the environment. Recent surveys have estimated that in fishery industry, as well as in other food production sectors, the utilization of by-products will experience a significant increase in the future. Also waste products revenue will be a key factor in maintaining long-term profitability of industrial food processing.
By-products are important sources of aminoacids, peptides, and proteins; the latter can be converted by enzymatic hydrolysis to value-added peptides and applied to improve and upgrade the functional and nutritional properties of nutraceuticals. Possible health beneficial effects of peptides relate to nutrient uptake, immune defense, opioid, antioxidant and antihypertensive activities, holding promise for use of the byproducts as functional food ingredients, in the production of dietary formulations.
In this research, Yellowfin tuna (*Thunnus albacares*) meat is used as a model for fish processing by-products, as it is a large epipelagic species widely distributed in the tropical and subtropical waters of the major oceans; it represented the starting material for protein extraction and analysis. As first experimental step, much effort was put on the development of LC-MS/MS based analytical methods capable to afford adequate selectivity and sensitivity for the determination of proteome samples, as an outcome of the enhanced separation efficiency and high mass resolution.

The authors thank Shimadzu and Merck KGaA for the continuous support.

O1 EAC3

SPECIATION ANALYSIS OF THALLIUM BY SIZE EXCLUSION/ION EXCHANGE LIQUID CHROMATOGRAPHY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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Metallic pipes in drinking water (DW) distribution networks are able to retain some elements over time that may be subsequently released into the water. In September 2014, researchers from the Department of Earth Sciences (University of *Pisa*, Tuscany, Italy) reported the presence of thallium in water samples collected from *Pietrasanta* DW wells (*Lucca*, Tuscany, Italy) at concentrations (up to 10 µg/L) much higher than US-EPA max contaminant level goal (2.0 µg/L).
We have developed an innovative extraction procedure to determine the thallium water-soluble fraction retained by contaminated pipe core samples. The target fraction is solubilized in acetic acid solution by means of consecutive sonication treatments (at least, five) carried out after changing the aliquot of the extractant. In order to characterize the inorganic species solubilized in acetic acid, first we performed speciation analyses of Tl(I)/Tl(III) released by Tl_2O_3 using anodic stripping voltammetry (polarography with a dropping mercury electrode (DME)). The main difficulty of speciation analysis of thallium lies in extremely low concentrations of Tl (III) in comparison to Tl (I), which is the dominating form of thallium in DW samples.
To confirm and optimize the trends obtained by voltammetry, in this study, an inductively coupled plasma mass spectrometer (ICP-MS) was used as a liquid chromatographic detector for the speciation analysis of thallium. Tl(I) and Tl(III) – diethylenetriamine pentaacetic acid (DTPA), were separated by using two separation mechanisms, anion exchange chromatography and size exclusion chromatography (SEC), with 10 mmol ammonium acetate as eluant.
From a solution of Tl_2O_3-DTPA, the effect of the concentration of the eluent, the pH of the solution and the Tl_2O_3-DTPA ratio on the retention times of Tl(I) and Tl(III) was evaluated. The chromatograms show good separation of the Tl peaks at each pH and hence for the differently charged Tl(III)–DTPA complexes. Therefore, whatever the charge, Tl(III) complexes do not co-elute with Tl(I) using SEC, because the molecular size is always greater than the Tl(I) ion, which clearly results in their earlier elution from the SEC column.
The proposed method enables the separation and determination of both Tl species. The method is fast, simple, accurate and interference-free.

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CHARACTERIZATION OF VOLATILE ORGANIC COMPOUNDS OF ESSENTIAL OILS PRODUCED IN TRIESTE KARST AREA BY HS-SPME-GC-MS AND COMPARISON WITH AN ELECTRONIC NOSE DISCRIMINATION POTENTIAL FOR QUALITY CONTROL PURPOSES

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According to the Commission of the European Pharmacopoeia [1] an essential oil is an "*odorous product, usually with a complex composition, obtained from a botanically defined plant raw material by steam distillation, dry distillation, or a suitable mechanical process without heating Essential oils are usually separated from the aqueous phase by a physical process that does not significantly affect their composition*".
Essential oils can be constituted by a variety of volatile organic compounds such as terpenes, alcohols, aldehydes, ketones, phenols, esters, ethers, oxides, peroxides, furans, organic acids, lactones, and sulfur compounds. Factors such as climate, soil, genetic features, and cultivation techniques greatly influence their composition in different plant species [2].
Since there is a growing interest in the use of essential oils as e.g. antimicrobial, insecticide, antiseptic, antifungal, and analgesic activities, aromatherapy, and disease treatments, there is the need of quality control for safety use starting from the raw material throughout the production process. Moreover essential oils can also be produced from different chemotypes which provide distinct chemical entities within the same botanical species [3].
Different chemotypes of the same species can show different activities in relation with the purpose and, in some cases a specific chemotype can contain toxic substances thus the use have to be avoided. The differences in chemotypes can be associated e.g. to the geographical area, the plant harvesting period and the vegetation environment [4]. Therefore there is the need of an extended characterization for quality control.
The aim of this study is the qualitative chemical characterization of essential oils produced in Trieste Karst area. Essential oils produced starting from thirteen plant species has been characterized by HS-SPME-GC-MS.
Nearly 280 compounds have been identified by a mixed use of: matching with mass spectra databases, Linear Retention Index and standard compounds.

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N	t.r. (min)	Compound	CAS	L.R.I.	Identification
1	5,7776	3-Pentanone 2,2-dimethyl-	564-04-5	839	LRI
2	5,9647	Butanoic acid 2-methyl-ethyl ester	7452-79-1	847	LRI
3	6,035	Cyclohexane ethylidene	1003-64-1	850	MS
4	6,0583	3-Octen-2-ol (E)	57648-55-2	851	MS
5	6,0816	Butanoic acid 3-methyl-ethyl ester	108-64-5	852	LRI
6	6,1285	Cyclopentane 1,1-dimethyl-	1638-26-2	854	MS
7	6,2454	2,4-Nonadiene (E)-	56700-78-8	859	MS
8	6,2454	Ethanone 1-(2-methyl-1-cyclopenten-1-yl)-	3168-90-9	859	MS
9	6,4561	1-Hexanol	111-27-3	868	LRI
10	6,713	1-Butanol 2-methyl- acetate	624-41-9	878	LRI

Figure 1. Part of the compound identification table (overall = 280 compounds)

73 compounds were found to be useful in identifying differences between species. Moreover we identified possible compounds related to the "Karst" chemotype comparing the results with those present in scientific literature.

N	t.r. (min)	Compound	CAS	C1	C2	C3	C4	C5	C6	C7	C8	C9
1	5,7776	3-Pentanone 2,2-dimethyl-	564-04-5						x			
2	5,9647	Butanoic acid 2-methyl-ethyl ester	7452-79-1		x							
3	6,035	Cyclohexane ethylidene-	1003-64-1				x					
4	6,0583	3-Octen-2-ol (E)	57648-55-2			x						
5	6,0816	Butanoic acid 3-methyl-ethyl ester	108-64-5		x					x		
6	6,1285	Cyclopentane 1,1-dimethyl-	1638-26-2				x					
7	6,2454	2,4-Nonadiene (E)-	56700-78-8			x						
8	6,2454	Ethanone 1-(2-methyl-1-cyclopenten-1-yl)-	3168-90-9				x					
9	6,4561	1-Hexanol	111-27-3	x			x					
10	6,713	1-Butanol 2-methyl- acetate	624-41-9						x			

Figure 2. Part of the presence/absence table for essential oil comparison (overall = 73 compounds)

The essential oil VOC profiles have been also analyzed by a 32 sensors array electronic nose to correlate the sensor signals to the chemical characterization and evaluate the e-nose discrimination potential. The data have been elaborated by Principal Component Analysis.

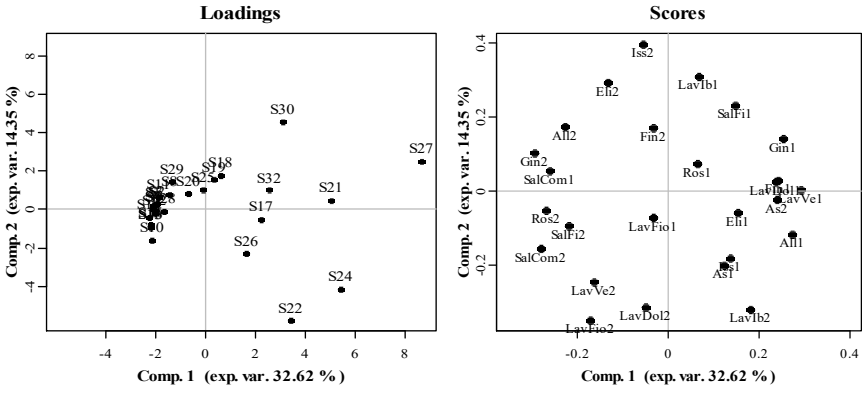

Figure 3. Principal Component Analysis of the electronic nose data.

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O3 EAC3

INTER-COMPARISON OF CARBON CONTENT IN PM_{10} AND $PM_{2.5}$ MEASURED WITH TWO THERMO-OPTICAL PROTOCOLS IN A MEDITERRANEAN SITE

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Carbonaceous fraction of atmospheric particulate matter (PM), generally ranging between 20% and 50% of PM mass [1], is usually classified into organic carbon (OC), elemental carbon (EC) and inorganic carbon (IC). The refractory light-absorbing component is called EC when measured by thermo-optical methods or equivalent black carbon (eBC) when measured with optical techniques. Quantification and characterization of carbonaceous particles are important to understand aerosol sources and their impact on air quality, human health, cultural heritage and climate. In fact, the European Directive 2008/50/EC requires the measurement of EC and OC in the $PM_{2.5}$ fraction of ambient PM in background areas.
A common widely used method is the thermo-optical transmittance (TOT), based on the differentiation between EC/OC according to their thermal and optical properties, using different protocols. Although a variety of inter-comparison studies of these standard approaches have been performed, the distinction between EC and OC is still a challenging problem [2], especially to maximize the comparability of results obtained in different sites. In addition, comparability of optically determined eBC and thermo-optically determined EC is also challenging because eBC and EC could vary by up to a factor 2 not only for the effect of thermal treatment but also for variability induced by size distribution, mixing state, and chemical composition [3,4,5].
In this work, a long-term campaign (January 2015-July 2016), was performed at the Environmental-Climate Observatory of Lecce (SE Italy, 40°20'8"N-18°07'28"E, 37 m a.s.l.), regional station of the Global Atmosphere Watch program (GAW-WMO) classified as an urban background site [6]. 369 daily PM_{10} and $PM_{2.5}$ samples were collected on quartz fibre filters (Whatmann, 47 mm in diameter, pre-fired for 2h at 700°C) using an automatic sampler (SWAM 5A Dual Channel Monitor, FAI Instruments srl). Simultaneous equivalent black carbon (eBC) concentrations, only for the PM_{10} fraction, were measured using a Multi Angle Absorption Photometer (MAAP Thermo Scientific, mod. 5012) operating at a wavelength of λ=670 nm using a constant mass absorption coefficient (MAC=6.6 m^2/g). Punches of 1.0 cm^2 were analysed for OC and EC determinations using a TOT method by a Sunset laboratory

carbon analyser (Sunset Laboratory Inc., OR, USA) with two different standard thermal protocols (NIOSH870 and EUSAAR2).

In summary, uncertainty of ~5% for TC and OC, compatible with the instrument manufacturer uncertainties, and larger uncertainty (~10%) for EC for both thermal protocols were found. Not statistically significant differences were found between the two protocols, for both size fractions, in TC and OC determinations. Contrarily, EC results obtained with EUSAAR2 were higher than that obtained with NIOSH870, with a difference of 19% and 33% for PM_{10} and $PM_{2.5}$, respectively. These values have a clear seasonal variability due to different combustion sources acting during cold (autumn and winter) seasons (road traffic and biomass burning). EC/TC ratios were larger for EUSAAR2 in both size fractions (23% for PM_{10} and 32% for $PM_{2.5}$), especially in the warm period (spring and summer). Differently, on average, OC/EC ratio derived from NIOSH870 was larger than obtained by EUSAAR2, being dependent on the medium-low temperature protocol.

SOC (Secondary Organic Carbon), estimated by EC-tracer method, has larger values during cold seasons, without differences between the two protocols used. The contribution of SOC to TC is essentially the same with both protocols and fractions, while SOC/OC is equal to about 51% (Figure 1) with no seasonality. Good correlation ($0.83<R^2<0.88$) was found between eBC and EC in PM_{10} even if eBC daily mean values were larger than EC measured with both protocols, as shown by the eBC/EC mean ratio, 1.62 for EUSAAR2 and 1.92 for NIOSH870 (Figure 1). The same trend was observed for both cold and warm periods.

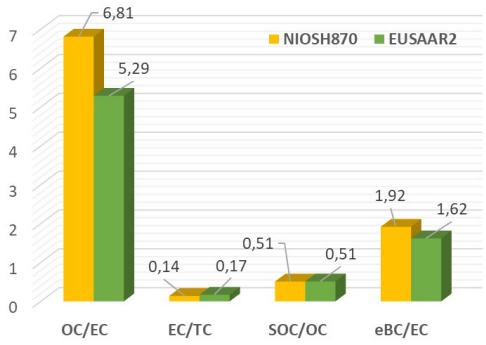

Figure 1. Comparison of some average parameters obtained with NIOSH870 and EUSAAR2 protocols.

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O4 EAC3

TRACE ELEMENTS IN LIGHT FUELS: DETERMINATION BY TOTAL REFLECTION X-RAY FLUORESCENCE AND COMPARISON WITH ICP-MS

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The presence of elemental impurities in fuels is a key topic with important consequences in several fields. They may adversely affect the performances of engines and of the catalytic converters employed for the abatement of noxious gases in the exhaust. As an example, lead is a well-known poison for automotive catalytic converters [1]. Moreover, they may contribute to the contamination of the environment, primarily air but also, on the long term, waters and soils: in this regard, the presence of sulfur causing air pollution by sulfur oxides and the consequent acid rain is highly representative (e.g. [2]).
Trace elements in fuels may originate from several sources [3]: natural origin, i.e. originate from the crude oil the fuel was distilled from, accidentally introduced together with additives and unintentionally present (catalyst residues, corrosion phenomena). Accordingly, the determination of trace elements in fuels is of the utmost importance in several fields, ranging from quality control to regulatory issues (e.g. emission control). Traditionally, atomic spectrometric techniques have been employed to this aim [4]: these techniques typically require a pretreatment step involving either sulfate ashing, microwave digestion or dilution in solvents. Here we propose a fast, direct and sensitive method for the quantification of V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As and Pb at trace level in light fuels, namely gasoline, racing and jet fuels based on Total Reflection X-Ray Fluorescence. A straightforward sample treatment procedure based on on-site enrichment, i.e. evaporation of high sample volumes (80 to 300 µL) achieved very low detection limits below 1.5 ng/g for the investigated analytes. Optimization also involved the selection of the best internal standard for quantification in terms of element, its complex solubility and solvent miscibility with the fuels. Validation on real samples was performed by comparison with data obtained by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

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O5 EAC3

APPLICATION OF A CHEMOMETRIC APPROACH TO A PRELIMINARY GEOCHEMICAL CHARACTERISATION OF THE TIMAVO/REKA RIVER MOUTH

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Accumulation of contaminants and their potential mobility represent two of the main environmental issues facing marine coastal environments. Sediments often act as "reservoirs" of contaminants including potentially toxic trace elements but they can also be considered a secondary source due to remobilisation processes at the water-sediment interface, which can affect the water quality [1].
The Timavo River, whose source is in Croatia, flows on flysch terrains in Slovenia (where it is called the Reka) before sinking into the Karst Plateau at the Škocjan Caves. After an underground path of approximately 38 km the river re-emerges from several springs 2 km from the sea in the northwest sector of the Gulf of Trieste, the site of an industrial area.
This work aims to provide a preliminary geochemical characterisation of the Timavo river mouth, focusing on the occurrence and distribution of trace elements (Al, As, Ba, Cd, Co, Cu, Cs, Cr, Fe, Hg, Mn, Ni, Pb, V and Zn) in different environmental matrices. For this purpose, water (surface and bottom) and sediment samples were collected from five sites in the estuarine system of the Timavo River. In addition, continuous salinity, temperature and turbidity vertical profiles were recorded in order to identify the water masses and the main physico-chemical parameters were measured *in situ*. Size fractionation was performed using vacuum filtration to isolate the suspended particles from the dissolved fraction.
The hydrodynamic conditions showed a sharp halocline even in the innermost sector of the study area, attesting to the evident and permanent salt-wedge intrusion. Due to cooling waters from a thermal power plant, particularly warm waters were found in the mixing and bottom layers of the water column and the reductive conditions measured at the bottom in the innermost sector of the study testify to potential anoxia at the sediment-water interface.
Principal Component Analysis (PCA) was employed on chemical and grain-size analyses in order to reveal disparities among the different sectors of the estuarine system. The surface sediments were found to be enriched in trace elements whose concentrations often exceed the Italian regulatory threshold limits, in particular in the innermost sector. Furthermore, the PCA results also demonstrate that several trace elements are strongly linked to the silty-clay

fraction which prevails in the innermost sector of the study area. The only exception is represented by Cr and Ni since the Timavo River contribution can be clearly identified as the primary source due to alteration processes of Cr-spinel enclosed in the bauxite veins found along its underground river path [2]. The reductive conditions found in the innermost sector appear to be responsible for dissolution processes, increasing dissolved trace elements in the water column. Conversely, oxidative conditions prevail in the external sampling sites. Here, oxidation and precipitation processes lead to trace elements partitioning in the suspended particles.

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O1 SS4

CHARACTERIZATION OF SPIRULINA MICROALGAE POLAR LIPIDS PROFILE COMPARED TO ENRICHED EXTRACT AND COMPREHENSIVE IDENTIFICATION WITH LIPOSTAR

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Microalgae species are characterized by different bioactive components, such as lipids, proteins, carbohydrates and pigments. Nowadays, polar lipids classes represent an important analytical target due to their bioactivity and biological functions. Until now, microalgae lipid profiling was mainly focused on free fatty acids and triacylglycerols, whereas information on the occurrence of glyco-, sulpho- and phospholipids is rather scarce [1]. In the present work, an optimized extraction procedure was employed in order to maximize lipids recovery using the *Arthrospira platensis*, also named spirulina microalgae. A solid-liquid extraction procedure was exploited, based on the use of a mixture CH_3OH:$CHCl_3$:H_2O. The hydroalcoholic phase was analyzed by Ultra High Performance Liquid Chromatography (UHPLC) coupled to high resolution tandem mass spectrometry (MS) followed by a bioinformatics procedure conducted by Lipostar, a comprehensive platform-neutral cheminformatics tool for lipidomics. A chromatographic evaluation, based on the type and concentration of mobile phase additives, gradients and pH of mobile phase, was carried out to separate the largest number of specific lipid classes with emphasis on glyco-, sulpho- and phospho- lipids under MS-compatible conditions. The chromatographic parameters were calculated both in negative mode, for sulfolipids and phospholipids, and in positive mode, for glycolipids, to obtain the best separation on a standard mixture. Afterwards, in collaboration with software creators and developers, Lipostar was implemented to improve the identification of phosphoglycerolipids and the identification of glycosylmonoradyl- and glycosyldiradylglycerols classes. Finally, the polar lipid extract of spirulina microalgae has allowed the identification of 205 lipids [2]. Afterwards, cause to the relevant abundance of sulfolipids in this matrix, an enrichment procedure based on graphitized carbon black (GCB) to enhance the specificity of the analysis about this specific lipid classes were performed. To this scope, after a solid-liquid extraction, sample was treated with GCB and analyzed with the proper UHPLC and MS conditions. A comparison between the GCB enrichment protocol and a no enrichment approach was also carried out to establish the enrichment efficiency in term of recovery and number of identifications. With a reliable lipid structure assignment conducted by Lipostar, the identification of 199 sulfolipids and only 60 sulfolipids in the no enriched sample was identified. This approach allowed us to characterize sulfolipids profile, identifying the highest number ever reported for the *Arthrospira platensis* species. Finally, a method validation in terms of precision, accuracy, recovery and limit of quantitation and

detection and a semi-quantitative analysis was carried out to characterize its sulfolipids profile and to estimate the concentration levels.

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O2 SS4

IMPACT OF HIGH POROSITY SILICA ON ZWITTERIONIC TEICOPLANIN-BASED COLUMNS FOR *ULTRA-HIGH PERFORMANCE CHROMATOGRAPHY*

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The research in the area of enantioselective Ultra High Performance Chromatography (eUHPC) is continuously focused on achieving higher efficiencies and, at the same time, faster analyses. In this work, a novel Chiral Stationary Phase (CSP) was prepared by covalently bonding the teicoplanin selector (TE_A2-2) on Halo 2.7µm 160Å Superficially Porous silica Particles (SPPs) by employing an already known synthetic procedure allowing to obtain a zwitterionic teicoplanin based CSP, which was used to produce the UHPC-FPP-Titan-Tzwitt CSP based on 1.9 µm 120Å Fully Porous monodispersed silica Particles (FPP) and UHPC-SPP-Halo90-Tzwitt CSP 2.0 µm [1-3]. These CSPs were packed into columns (L.: 50 and 100 mm, I.D.: 4.6 mm) and were characterized in terms of permeability, efficiency and thermodynamic under HILIC condition. van Deemter curves were used as main instrument for the kinetic performance evaluation. The UHPC-SPP-Halo160-Tzwitt 2.7 µm showed excellent efficiencies on both achiral (>323,000 theoretical plates/meter, *N*/m; h_r: 1.14) and chiral analytes (>240,000 *N*/m; h_r: 1.53), proving the high potential of this CSP from the kinetic point of view also in comparison to the UHPC-SPP-Halo90-Tzwitt CSP 2.0 µm and UHPC-FPP-Titan120-Tzwitt CSP 1.9 µm. Furthermore, taking into account the thermodynamic viewpoint, on the one hand, the UHPC-SPP-Halo160-Tzwitt 2.7 µm exhibited significantly smaller retention factors (*k'*) in comparison to those observed on the two sub-2µm UHPLC columns (as a consequence of the lower selector loading on the silica). On the other hand, the SPP-Halo 160Å column showed the best resolution power ($Rs/t_{r,2}$) thanks to its enantioselectivity values because of the larger selector density on the silica matrix. In conclusion, in this study we present the potential of the use of high-porosity SPP silica particles in the UHPLC chiral field opening an interesting scenario in this area.

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O3 SS4

FLUORESCENCE LABELLING FOR THE STUDY OF PHENOLIC GROUPS DISTRIBUTION IN TECHNICAL LIGNIN AS A FUNCTION OF THE MOLECULAR WEIGHT

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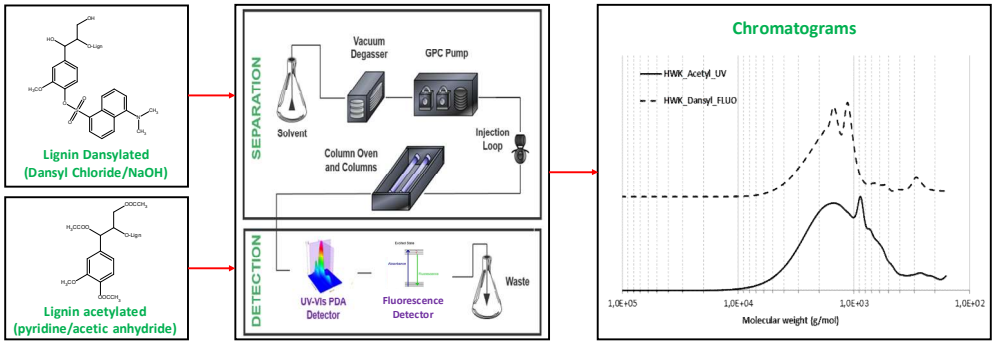
Lignin is a three-dimensional polymer and the second most abundant natural polymer after cellulose. With hemicelluloses, those two biopolymer constitute the so-called lignocellulosic materials. The lignin structure consists of three phenolic monomer, p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) which are linked through ether and carbon-carbon bonds resulting in heterogenic and irregular macromolecules [1]. Moreover, to separate lignin from the lignocellulose compound, intermolecular linkages are broken and modified during the pulp and paper and/or biorefinery processes. Molecular weight as well as the functional group (hydroxy, methoxy, carboxyl) are highly affected by the separation process [2]. The resulting technical lignins are indeed complex, irregular, polyphenolic compounds: this heterogeneity is the main drawback for a reliable lignin valorisation, one of the most important point in the field of biorefinery [3]. This is the reason why recently many papers were focused on lignin fractionation [4, 5]. In general, from an analytical point of view, the understanding of technical lignins is set back by the common analysis approach putting together fragments with known structural features and perhaps some newly identified motifs, and complementing this by analysing many functional groups. As reported by Potthast and co-worker, however, we have not yet arrived at a stage where we can state that we comprehend the whole picture of this fascinating molecule [6]. In order to overcome this problem and to increase our understanding of the structure of technical lignins, an alternative analytical approach have been set up. In particular, the approach in based on the fluorescence labelling of lignin with dansyl chloride, selectively and quantitatively on phenolic groups. Phenolic groups have been selected because: i) they are the main functional groups on lignin; ii) they are ease to modify; iii) in term of valorisation they are the more valuable chemical group: they have antioxidant properties and they can be converted in other reactive functionalities. The selectivity and the yield of the labelling reaction was checked by ^{31}P-NMR. Then the fluorescence labelled lignin was submitted to Gel Permeation Chromatography (GPC) coupled with a fluorescence spectrometer. The comparison of the GPC profile of a simply acetylated lignin sample (detect by UV, bearing the information related to the biopolymer molecular weight distribution) with the GPC profile of the same lignin sample, but dansyl labelled on phenolic groups, give us the opportunity to obtain information about the distribution of this important functionality as a function of the molecular weights. After data elaboration (from GPC comparison) and total phenol content measurement (by ^{31}P-NMR) it is possible to defined the phenol concentration at different molecular weight ranges. In Table 1 are reported the phenol

contents of different technical lignins at different molecular weight ranges. This information is extremely valuable for many reasons: i) it is possible to understand how the extractive process affect the lignin structure; ii) it is possible to evaluate which fraction of lignin is more suitable for one specific application. In future perspectives, this approach could be apply to other chemical group such as alcohols and carboxylic acid. In this view, we can obtain interrelations between functional groups and the molecular weight ranges, for different lignin sources. This could help to establish for technical lignins structure-property-application relationships (SPARs) that are required for any future large-scale application.



MW range (g/mol)	SWK (mmol/g)	HWK (mmol/g)	SG (mmol/g)	WS_{SE} (mmol/g)	MS_{Acid} (mmol/g)
20000-10000	0.00	0.07	0.00	0.00	0.04
10000-5000	0.99	1.41	0.14	0.36	0.29
5000-3000	2.67	2.39	1.03	1.02	0.76
3000-2000	4.07	2.77	2.14	1.65	1.37
2000-1000	6.35	3.63	4.78	2.57	1.44
1000-500	3.70	1.14	4.60	2.28	1.17
500-160	4.76	2.24	3.30	1.82	0.52

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O4 SS4

POTENTIAL OF ON-LINE LC-FTIR HYPHENATED TECNIQUE AS A RELIABLE TOOL FOR IDENTIFICATION

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LC-IR or HPLC-IR is a hyphenated technique developed from the coupling of an LC and the detection method infrared spectrometry (FTIR). While, chromatography on LC column based on partially porous particle technology, affording high peak capacity and excellent reproducibility of peak retention times/areas, is one of the most powerful separation techniques available today; FTIR is a good spectroscopic technique for the identification of organic compounds, in particular for the composition and structure of isomeric compounds, providing valuable information about local configuration of atoms in molecules.
Hyphenated technique based on HPLC-FTIR can fill a gap in identification by mass spectrometry (MS), in discriminating geometrical isomers and diasteromers and closely related molecules and can be effective whenever unambiguous identification of low-level sample constituents is required. The more successful coupling of LC and FT-IR is accomplished by solvent elimination prior to IR detection; this method involves an interface that evaporates the eluent and deposits the analytes onto a medium compatible with FT-IR detection.
The FTIR system here presented is connected to the outlet of a LC column, through a solvent removal interface, and deposits eluants as a continuous track of sample on a rotating IR transparent ZnSe disk. The built-in interferometer simultaneously captures a set of time-ordered IR spectra from the deposit track, allowing for good quality spectra to be obtained on nanogram amount of analytes. The obtained spectra is independent of the solvents used so there are no spectral restrictions on the solvents used as eluents. In addition, spectra obtained using ZnSe closely resemble conventional KBr disk transmission spectra and ATR, and thus spectral libraries and search programs may be used for identification purposes, enforcing the reliability of IR as detection technique.
In this study, HPLC-FTIR integrated system has been employed for the separation and identification of coumarins and furocumarins isomers and the best operating condition for

the revelation are reported. Whereas the separation of these compounds can be problematic due to their similar polarity, chemical structures and equal molecular weight, a series of experiments performed changing both chromatographic and spectroscopic parameters allowed the detection and the chemical identification trough library matches.

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O5 SS4

A NOVEL MOLECULARLY IMPRINTED MAGNETIC MATERIAL FOR THE ACCURATE DETERMINATION OF ZEARALENONE MICOTOXIN IN COMMERCIAL FLOUR SAMPLES BY HPLC-MS/MS

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Zearalenone (ZEN) belongs to the class of mycotoxins mainly produced by Fusarium fungi and generally occurs in cereal grains. ZEN shows oestrogenic activity and, due to its persistency in the food chain, it constitutes a risk to human and animal health thus requiring monitoring actions. For this reason, analytical methods for its accurate determination are required and pre-concentration and clean-up become fundamental aspects to take into account. Molecularly imprinted polymers (MIPs) are widely used as selective or specific materials for sample extraction and enrichment; some examples can be found about MIPs prepared for the extraction of ZEN from different matrices, mainly obtained by bulk polymerization. In this framework, a great improvement can be brought by employing MIP particles in the nanometre dimension range, which provide extensive contact with the sample, hopefully improving analyte recovery. The present work deals with the preparation of MIPs nanoparticles, designed for ZEN binding, and their application to ZEN extraction from different flour samples for the consequent analysis by liquid-chromatography coupled to mass spectrometry (LC-MS). The material was prepared by an innovative polymerization approach, which involves multiple steps: a controlled aggregation of magnetite particles; a coating and functionalization of the aggregates with tetraethylorthosilicate and vinyltriethoxysilane; finally, the formation of an external polymer shell, synthesized in the presence of a dummy template molecule, in order to obtain specific cavities for the interaction with ZEN. The dummy template molecule (quercetin) was selected for its structural similarity to the analyte of interest and was preferred to the use of the real template to avoid problems of contamination due to residual ZEN in the material. The dimension of the MIP nanoparticles was monitored during the entire synthesis, which led to a final average diameter of 900 nm. The corresponding non-imprinted polymer (NIP) was prepared as well, for selectivity comparison, and both materials were characterized by scan electron microscopy (SEM), showing a similar morphology. The thermodynamic and kinetic behaviours of both ZEN-MIP and NIP were investigated by means of static and dynamic adsorption tests, demonstrating a higher adsorption capacity of the imprinted nanoparticles and quick kinetics, with equilibrium reached after a 10 min contact. The ZEN-MIP material had 55-fold more affinity for ZEN than the NIP material, which supported its potential for ZEN pre-concentration from complex food matrices. Therefore, a simple protocol was

proposed for the pre-treatment of flour samples, slightly modifying a previous method developed in our laboratory [1]. After evaporating an acetone extract of the flour sample, the residue was reconstituted in a suitable solvent and put in contact with the MIP material for 30 min; afterwards, the MIP was washed, to remove interferents physically adsorbed onto the surface, and two elution steps were performed to recover ZEN. The samples were analysed by HPLC-triple quadrupole MS in multiple reaction monitoring, to enhance specificity and sensitivity. Excellent recovery (89±11 %) and matrix effect (101±2 %) were observed for the compound of interest, by testing three fortification levels on blank samples. The method detection and quantitation limits were 44 and 140 pg g^{-1} respectively, while repeatability was assessed by evaluating intra-day and inter-day relative standard deviations (7% and 10%, respectively). The proposed method was compared with a simple solid-liquid extraction and with a clean-up by classical solid phase extraction, showing a significant improvement in the observed matrix effect by using the MIP material. Finally, seven flour samples of different cereals were analysed, demonstrating the suitability of the method to ZEN accurate determination in complex matrices.

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O1 EC

LABORATORY AND FIELD PROCEDURES FOR THE DETERMINATION AND SPECIATION OF MERCURY BY ANODIC STRIPPING VOLTAMMETRY

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The availability of reliable and sensitive procedures for the determination and speciation of mercury is of great interest in several fields, such as environmental monitoring, food safety and clinical toxicology, owing to the potential toxicity of this metal, even at low concentrations. The most common analytical techniques for mercury quantification are cold vapour or fluorescence atomic absorption spectrometry and inductively coupled plasma mass spectrometry. Furthermore, direct mercury analysis can be carried out with devices able to automatically perform both sample decomposition and analyte detection by AAS [1]. An alternative approach for mercury determination is the use of electrochemical techniques, which are sensitive, versatile, inexpensive and suitable for on-site measurements with portable instrumentation. Most procedures rely on anodic stripping voltammetry (ASV), but also stripping chronopotentiometry, potentiometry and pulsed amperometry coupled with high performance liquid chromatography (HPLC) have been adopted. As to ASV, the most suitable working electrode (WE) material for mercury determination is gold, in different forms: solid, microwire, fiber, ultramicroband array, film (deposited on conventional or screen printed carbon substrates), nanoparticles. Gold WEs are used without modification or modified with different reagents, such as ligands or thiols. Other electrode materials used for mercury are glassy carbon, carbon paste, boron-doped diamond, platinum, modified with organic molecules, polymers or other modifiers [2].
The knowledge of mercury speciation is important, since organometallic forms, deriving from methylation of inorganic mercury, are more toxic than the latter. Speciation techniques for mercury are usually based on extraction or chromatographic separation coupled with spectrometric or (less commonly) electrochemical detection.
Our research group has been studying mercury determination and speciation since 2008. We have developed analytical methods for quantifying the total concentration of this element by ASV with a commercial solid gold electrode (SGE) and with a home-made nanoparticle-modified glassy carbon electrode (AuNPs-GCE). The advantages of AuNPs are their electrocatalytic properties, their large surface area, which ensures better mercury preconcentration and lower detection limits, and the renewable surface. The AuNPs-GCE has been applied to the determination of mercury in different matrices, such as drinking waters, sediments, food and pharmaceuticals.

Finally, we have focused our attention on fish [3], which accumulate relevant concentrations of mercury in their tissues and thus, can represent a major dietary source of this element for humans (Figure 1). We have developed a determination and speciation protocol able to distinguish between inorganic mercury (HgIN) and methylmercury (MeHg), the most commonly occurring methylated form of this element in natural waters. The protocol consists of the following steps: i) digestion of an aliquot of fresh fish with a mixture of HNO_3 and H_2O_2, followed by determination of total mercury (HgTOT) by ASV; ii) extraction of another sample aliquot with HCl, and treatment of the extract in a cartridge packed with a commercial resin modified with a ionic liquid (Patent Pending), which selectively retains HgIN; iii) elution of HgIN and analysis of the eluate by ASV; iv) calculation of MeHg by difference.

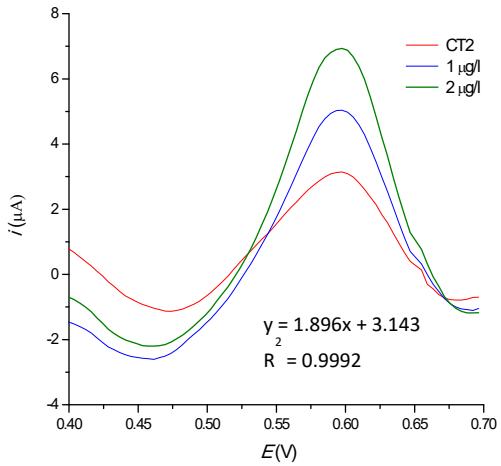

Figure 1. Voltammograms obtained for Hg quantification in a sample of canned tuna fish (CT2) with AuNPs-GCE

The protocol was tested on a certified reference material, Tuna Fish BCR 463 (98% recovery) and applied to the analysis of fish samples (canned tuna, swordfish, mussels, etc.) with two different approaches: i) microwave oven digestion and benchtop voltammetric analyzer; ii) digestion with a commercial food warmer and portable analyzer (Palmsens3), in view of on-site measurements. The LOQs in the fish-matrix were 0.5 μgL^{-1} for SGE and 0.1 μgL^{-1} for AuNPs-GCE, corresponding to 0.30 and 0.06 $mgkg^{-1}$ in the fresh sample. The samples were analyzed in parallel using direct mercury analyser (DMA) at the IZPLV: consistent results were obtained with the two voltammetric approaches and the DMA.
The proposed protocol is simple, inexpensive, and suitable for on-site analysis, allowing for the increase of controls on fish batches, thus reducing the risks to consumer health.

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O2 EC

ELECTROCHEMISTRY OF, AND ELECTROANALYSIS IN, CHIRAL AND INHERENTLY CHIRAL IONIC LIQUID MEDIA

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Ionic liquids ILs are attractive media for electrochemistry and electroanalysis, since, in addition to other useful properties, they provide both solvent and supporting electrolyte; moreover, they feature an extremely well defined structure at the interphase with a charged electrode, resembling a semisolid crystal or a bulk liquid crystal, extending for many layers, also standing in the presence of water traces, and possibly tunable by *in-situ* present additives. For this reason *chiral* ionic liquids CILs are surprisingly still nearly unexplored by electrochemists, in spite of appearing quite attractive, since they could transmit chiral information more effectively than chiral organic solvents or chiral supporting electrolytes.[1] In this context we have recently started a detailed investigation of *(a) bio-based chiral* ionic liquids and *(b) inherently chiral* ionic liquids ICILs, investigating both their physico-chemical and electrochemical features and their performance as media for chiral electroanalysis experiments. Our bio-based CILs feature cations with a building block of natural origin, including one or more localized stereocentres, from which their chirality arises (as in most so far available CILs) [2]. Instead in our ICILs chirality is intrinsic of the whole biheteroaromatic cation, which features a high torsional angle between two equal moieties, with a related energy barrier too high to be overcome at room temperature, so that the ICIL can be obtained in two stable enantiopure antipodes. Actually, while interesting but (at least so far) small are the chirality effects observed working in our bio-based CILs with localized stereocentres [2], large peak potential differences have been observed for the enantiomers of very different chiral probes in CV experiments in an enantiopure bulk ICIL. Very conveniently, impressive enantiodiscrimination is observed even using ICILs (or other related inherently chiral molecular salts, solid at room *T* but of easier synthesis) as low-concentration chiral additives in common achiral ionic liquids ILs [2]. Furthermore, similar impressive performances have also been observed dissolving in an achiral IL a thiahelicene-based additive, also inherently chiral, but uncharged and based on a different stereogenic element, *i.e.* a *helical* scaffold.

O2 EC

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O3 EC

ELECTROANALYTICAL CHARACTERISATION OF NITROSAMINES FOR THEIR POST-COLUMN AMPEROMETRIC DETECTION

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N-Nitrosamines (NAs) are carcinogenic, mutagenic and teratogenic substances that can occur in human diet and other environmental media [1]. In particular, NAs represent a serious contamination problem for meat and meat products subjected to technological processes [2].
Nitrosamines are traditionally analyzed by gas or high performance liquid chromatography coupled with thermal energy analyzer [3], mass spectrometric [4] or spectroscopic [5] detectors.
NAs show also electrochemical properties that make electrochemical techniques as good alternative methods for their determination [6-10].
In spite of the good sensitivity shown by these methods the main disadvantage is the poor selectivity that require the coupling with an efficient separation method. On the best of our knowledge only few attempts regarding the post-column polarographic determination of NAs at mercury electrodes were proposed [11-13].
In order to couple the electrochemical detection to a chromatographic separation it is essential to verify the electrochemical response of the analytes according to the composition of the various mobile phases used in chromatography.
Therefore, in the present work an extensive electroanalytical characterization of NAs (n-PYR, n-MOR, n-DEA, nDPhA) was carried out for the purpose of developing analytical methods based on post-column amperometric detection. Preliminary experiments were carried out by cyclic voltammetry to investigate NAs electrochemical behavior on Au, Pt and glassy carbon electrodes in typical mobile phases, and to select the best electroanalytical detection conditions. In addition, flow injection analyses were carried out in order to evaluate some performance parameters such as sensitivity, limit of detection and response stability.

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O4 EC

CHEMICAL AND ELECTROCHEMICAL PROPERTIES OF HYDROPHOBIC DEEP EUTECTIC SOLVENTS

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A Deep Eutectic Solvent (DES) is a eutectic mixture formed by a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA). The interactions between these components lower the melting point of the mixture with respect to those of the individual components, leading to a liquid phase at room temperature. DESs have been recently acknowledged to offer remarkable advantages over the common organic solvents thanks to their simple preparation, good biodegradability, low toxicity and low cost. These features make them suitable for large-scale production, being effective, greener alternatives to other solvents possessing some similar physicochemical properties, such as ionic liquids.
Hydrophobic DESs were recently introduced [1]; they are based on poorly water-soluble components, such as tetraalkylammonium salts, long chain carboxylic acids, menthol and lidocaine. An unprecedented study of the electrochemical properties of prototypical hydrophobic DESs [2], based on tetrabutylammonium chloride (TBAC) or Aliquat 336 and decanoic acid (DecAc) (1:2 molar ratio), is carried out, proving that these solvents are suited to be employed as electrolytic media to be used in the electrochemical frame.
Although the role of water content is largely underrated for both hydrophobic and hydrophilic DESs, we could demonstrate that it induces dramatic changes in DESs' physicochemical properties: a small water content is shown to be advantageous as it dramatically affects the electrical conductivity and the viscosity of the solvent, i.e. the parameters of fundamental importance in electrochemistry. Different preparation procedures were developed, leading to a DES with a desirable water content. In order to show the effect of water content, voltammograms of ferrocene in DESs with different amount of water were recorded (DES1 (0.014% w/w), DES2 (0.11% w/w), DES3 (1.70% w/w) and DES4 (2.4% w/w), as indicated in Figure 1). Figure 1 shows that the lower the water content of the DES, the larger the peak separation and the lower the current, which can be ascribed to the decreased conductivity, inducing ohmic drop, and to increased viscosity of the solvent, lowering the rate of mass transfer.

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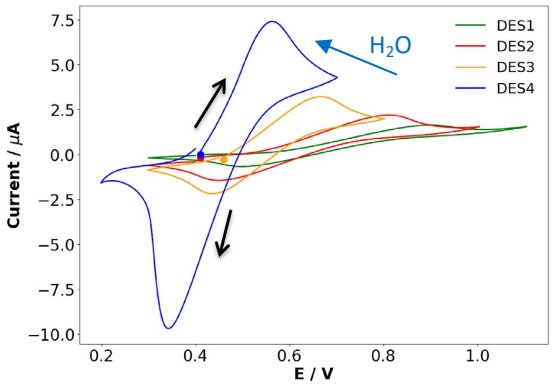

Figure 1. Electrochemical responses of ferrocene on a GC electrode employing four DESs based on TBAC and DecAc with different water content, 50 mVs^{-1} potential scan rate.

The use of hydrophobic DESs for the liquid-liquid extraction of metallic species from aqueous solutions was considered: a critical environmental pollutant, namely Cr(VI), has been successfully extracted from aqueous phase and the extraction can be easily checked by naked eye (Figure 2a). Cr(VI) has been also amperometrically detected in the DES phase (Figure 2b). This is necessary to the development of procedures for in-line amperometric sensing, as well as for electroremediation, both suited to be operated directly in DES.

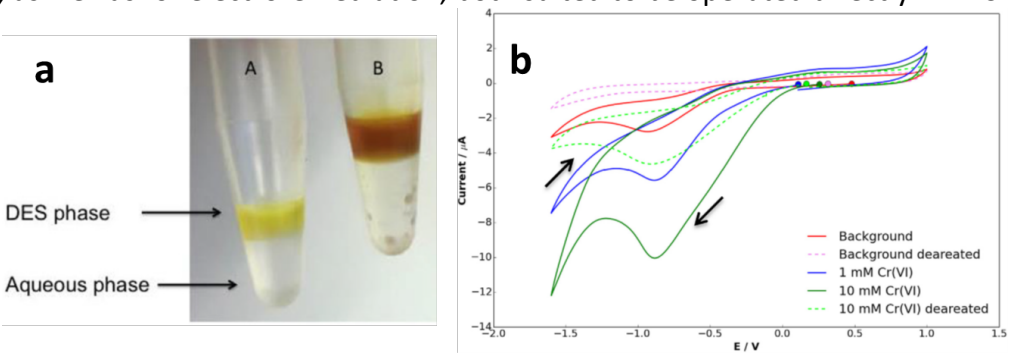

Figure 2. a: extraction in DES based on TBAC and DecAc from an aqueous solution of Cr(VI) species at 5 mM (A) and 50 mM (B); b: voltammetric responses of a GC electrode in the DES phase containing Cr(VI) at 10 mVs^{-1} potential scan rate.

However, the aqueous solubility of the components of some DESs could be significant, so we sought improvements to the DES formulation, to obtain a hydrophobic DES possessing a very low solubility in water, low viscosity and high electrical conductivity, thus allowing electrochemical investigations of the extracted species: linoleic acid is only efficient at low T, while cetrimonium chloride is too viscous to be used in electrochemical measurements. DecAc and Aliquat 336 were considered to be the most promising starting components; they also showed high thermal stability and resilience to oxidation by atmospheric oxygen.

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O5 EC

POTENTIOMETRY REVEALS THE INSTANTANEOUS PHOTOCATALYTIC RATE

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Since the pioneering applications of photocatalysis in the fields of energy and environmental remediation were proposed in the 1970s and 1980s, [1-3] many aspects of the photocatalytic process were thoroughly investigated, and several mechanistic studies appeared and tried to justify the peculiar behavior of the photocatalytic rate. [4,5] In particular, the basic model proposed by Minero at several reprises, [6-8] and validated in many different experimental conditions, [9] explained the overall photocatalytic process in terms of basic photocatalytic reactions like the charge carrier generation and their transfer and recombination as a second-order reaction. Usually the rate is evaluated through the measurement of the time evolution of the concentration of some target organic compounds with classical analytical techniques.
Following this basic model, the photocatalytic rate in the absence of back reactions is proportional to the holes concentration in the photocatalyst particles and surface concentrations of reactants. However, under steady state conditions and in the simplest case, the photocatalytic rate could be also measured from the photoelectron density. The latter can be assessed from the i-V plot of a semiconducting electrode, monitoring its open circuit potential. [10]
We propose that open circuit potential monitoring could be used to instantaneously evaluate the photocatalytic rate. We demonstrated that for substrates for which back-reactions can be ruled out i.e. the oxidized substrate is not prone to reduction, such as formic acid and alcohols like methanol and glycerol, the photoelectron density directly gives the photocatalytic rate, which linearly increases with the square root of the substrate concentration as predicted by the basic kinetic model. Additionally, increases (decreases) in the concentration of the oxygen results in the reduction (growth) of the photoelectron density, with the retention of the increasing trend of the rate with the square root of the substrate concentration. Moreover we demonstrate how open circuit potential responds to variations in incident photon flux, type and concentration of oxidant and substrate, and how the corresponding photoelectron densities could be translated into photocatalytic rates. Compared with the conventional measurement of substrate disappearance, the method here proposed is simpler and significantly faster. In fact it allows the assessment of the photocatalytic rate in different experimental conditions on the timescales of minutes and hours, when traditional measurement would require considerably longer times, and involve trained laboratory staff and dedicated analytical equipment. The method proposed is particularly suitable for the fast screening of different catalysts, substrates, and for the rapid assessment of the effects of the incident irradiation in terms of wavelength and intensity.
Conversely, when the substrate is prone to back reactions or the mechanism cannot be described by the simple model, as it was already suggested for phenol, we observed a more complex behavior. In these cases the dependence on the square root of the substrate concentration is not followed, and the photocatalytic rate is a complex function of the photoelectron density. Therefore, the open circuit potential gives complementary information with respect to the rate

data measured on phenol disappearance. Additionally, we observed contrasting effects of the oxidant concentration as a function of phenol concentration.

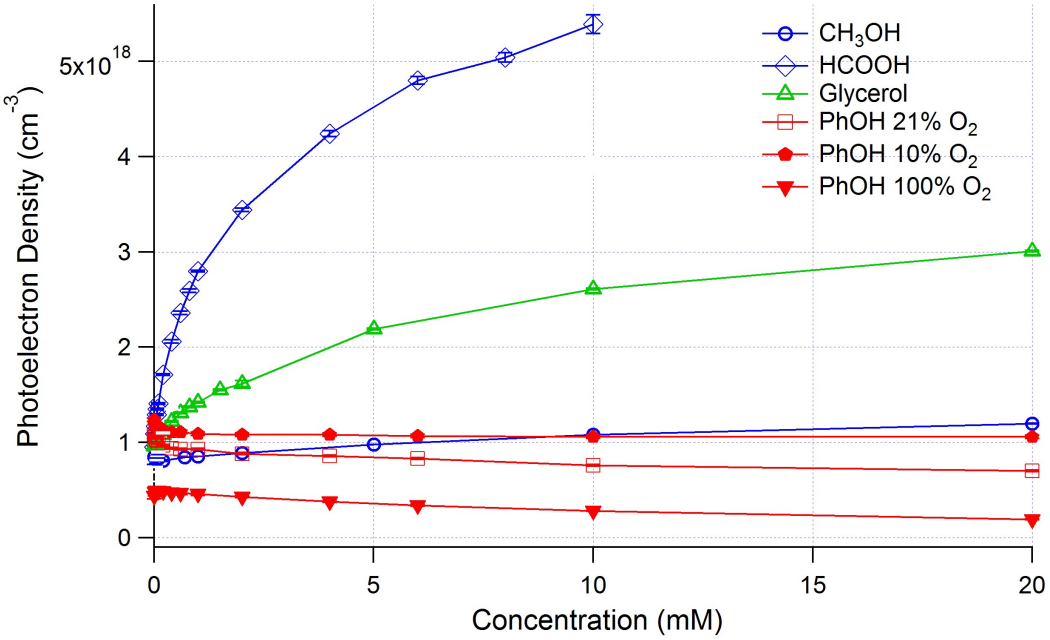

Figure 1. Photoelectron density as a function of substrate concentration.

The potentiometric method here presented is particularly suitable to gain mechanistic and kinetic insight, especially because it is possible to monitor the fast transients due do light ignition and discontinuation, and the additions and subtractions of substrate or oxidant, which cannot be studied with traditional means. The combination of the measure of open circuit potential with classical analytical techniques, in which the time evolution of the concentration of some target organic compound is evaluated, could be useful to assess the cases where the photocatalytic mechanism cannot be explained only in terms of basic photocatalytic reactions.

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O1 SES1

FRONTAL CHROMATOGRAPHY-ICP-MS: A NOVEL METHOD FOR FAST INORGANIC AS(III) AND AS(V) SPECIATION

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Analytical speciation methods for the trace determination of elements in different chemical forms have become increasingly important in the last decades. It is in fact well-known that the total concentration of elements cannot provide complete information about their impact on environmental and biological systems.
Routine speciation analysis are commonly based on the coupling of chromatographic separation techniques (e.g. HPLC) with a sensitive elemental detection system (such as ICP-OES or ICP-MS).
Since a large number of samples are usually analyzed with these routine methods (e.g. environmental samples, kinetic studies), the development of novel analytical protocols is favourable if an increase of the throughput together with a simplification of the required apparatus is delivered.
Since the partition coefficients of the species to be separated are usually markedly different, it is possible to simplify the instrumental apparatus by introducing a short column with a low number of theoretical plates instead of an HPLC system, with a consequent reduction of the analysis time. One advantage of this strategy relies on the low backpressure exerted by such short columns, which allows the feeding of the solution by a simple peristaltic pump (already present in all ICP-MS and ICP-OES instrumentation). Moreover, the exploitation of a frontal chromatography approach (i.e. a procedure in which the sample is fed continuously into the chromatographic bed) should avoid the presence of an injection valve, providing a further simplification of the overall system.
In spite of these considerations, the frontal chromatography still finds very little application for analytical purposes.
In light of this lack in the literature, we decided to make an investigation on the feasibility of a novel approach based on a frontal chromatographic system coupled with an ICP-MS to selectively analyze inorganic As(III) and As(V). Very preliminary data on this issue were already presented during the XXVII Congress of the Analytical Chemistry Division, while in this communication we would like to show the results concerning the fully developed method.
Briefly, complete frontal chromatograms can be recorded in around 160 seconds (Figure 1a) thanks to the introduction of a short column (60mm-long cartridge loaded with a strong anionic stationary phase) placed between the autosampler and the nebulizer. As(III) and As(V) are sufficiently well separated using this frontal chromatographic system, despite the low number of theoretical plates provided by the column.

O1 SES1

Multivariate and univariate methods were explored to accurately quantify As(III) and As(V). In particular, very good quantifications (relative errors in prediction under 3%) up to 135 ppb were obtained using PLS regression (Figure 1b-c). Additionally, LOD of 0.17 ppb for As(III) and 0.22 ppb for As(V) were estimated.
The actual time of analysis can be further reduced because accurate quantifications can be done using only the first 120 seconds of the frontal chromatograms. The simplification of the instrumental setup allows also to avoid time-consuming operations related to the employment of an injection loop (required for any HPLC-based method). Therefore, the resulting time of analysis is significantly lower than the ones reported in the literature for the fastest HPLC-ICP-MS inorganic As speciation methods [1].

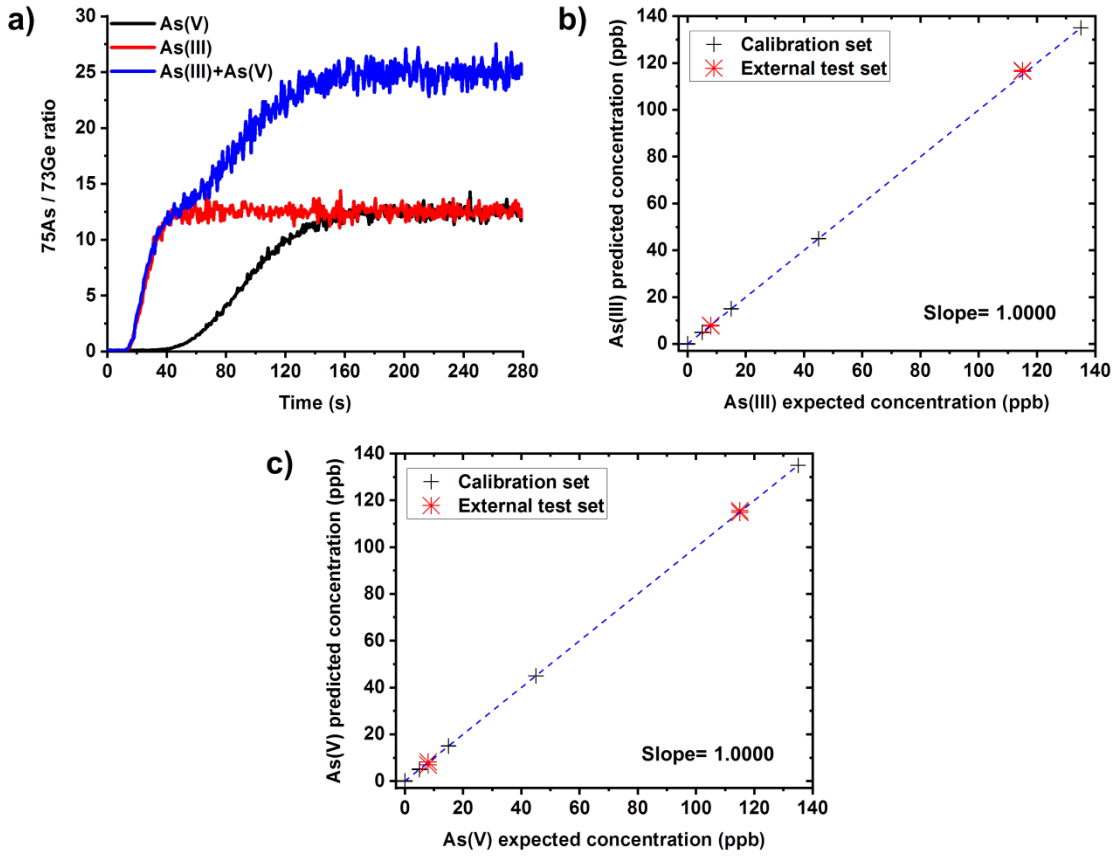

Figure 1. (a) Frontal chromatograms of a 135 ppb As(V) solution (black line), 135 ppb As(III) solution (red line) and a mixture solution containing 135 ppb of both As(III) and As(V). In all the measurements Ge was used as internal standard. (b-c) Expected vs. Predicted concentration plot for As(III) (Figure b) and As(V) (Figure c) obtained by PLS regression.

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O2 SES1

ADSORPTION OF CALIXARENE-BASED SUPRAMPHIPHILES AT THE SOLID-LIQUID INTERFACE MONITORED BY QCM-D

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Surfactants or amphiphiles are molecules that consist of a polar or ionic hydrophilic head group and a hydrophobic tail, often a long-chain aliphatic hydrocarbon group. Surfactants can be classified as anionic, cationic, nonionic and amphoteric based upon the nature of the hydrophilic head. Surfactants have widespread applications in industry as emulsifiers, foaming agents, wetting agents, dispersants and detergents. Pharmaceutical and biotechnology industries use surfactants for a variety of applications including stabilization of protein therapeutics.

Amphiphilic surfactants do not feel "at ease" in any solvent, be it polar or non-polar, since there is always one of the groups that does not like the solvent environment. Thus, these molecules do have strong tendency to migrate to interfaces or surfaces to orient themselves [1].

Self-assembly, making use of amphiphiles as building blocks, is an important topic in supramolecular chemistry. Besides the vast amount of synthetic conventional amphiphiles, there has been great interest in supramolecular amphiphiles (supramphiphiles) in which noncovalent interactions are used to promote their formation. Interactions between the hydrophilic and hydrophobic components may result from hydrogen bonding, electrostatic attraction, coordination interactions, charge transfer, π–π stacking and *host–guest* interactions [2, 3]. These systems can aggregate to form micelles, bilayers, vesicles.

In the present communication we report on interface adsorption studies of supramphiphiles formed by a p-sulfonatocalix[4]arene (*host*) and cationic *guests* that differ for the length of the hydrophobic tail or for the polar head; the process is monitored by Quartz Crystal Microbalance with Dissipation monitoring (QCM-D). Deposition of a thin, rigid and uniformly distributed layer on the quartz surface causes a decrease of the crystal oscillation frequency; however, when a soft or thick layer is bound to the crystal, dissipation changes [4].

The study at the solid-liquid interface compares the absorption features of conventional amphiphiles [5,6] with those of supramphiphiles. The adsorption of both conventional and supramolecular surfactants is strongly affected by the length of the apolar tail and, partially, by the nature of the polar head. Although supramolecular systems have critical micellar concentration (CMC) values much lower than the analogous uncomplexed surfactants [7], the absorbed amount at the interface is somehow comparable thus highlighting the key role played by the calixarene scaffold. Indeed, absorption isotherms may be satisfactorily obtained also when dealing with fairly diluted surfactant solutions. Furthermore, the presence of the calixarene moiety inhibits the "bulk" effect observed for surfactants with

large CMC value. Finally, kinetic adsorptions curves revealed significant insights on the rate as well as the mechanisms of the adsorption processes.

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O3 SES1

ACID-BASE PROPERTIES AND BINDING ABILITY OF AN ASPARTIC ACID DERIVATIVE OF 3-HYDROXY-4-PYRIDINONE TOWARDS BIOLOGICAL RELEVANT METAL CATIONS

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This contribution is the result of a speciation study in aqueous solution of a 3-hydroxy-4-pyridinone amino-acid derivative in the presence of two divalent metal cations of biological relevance, at different experimental conditions.
The 3-hydroxy-4-pyridinones (3,4-HP) are a class of compounds, derivatives of deferiprone, developed in view of applications in metal chelation therapy and for the detoxification of human body from *hard* metal cations (Fe^{3+}, Al^{3+}, etc.), because of their effectiveness at biological conditions, lows costs, oral activity, absence of toxicity and of side effects. They are featured by an aromatoid *N*-heterocycle with a hydroxyl and a ketone groups in *ortho* position, which confer them a strong affinity towards divalent and trivalent metal cations [1-3].
The ligand under study has a 3,4-HP core *N*-functionalized with an aspartic acid moiety. Its acid-base properties, previously studied at I = 0.15 mol L^{-1} in $NaCl_{(aq)}$ and T = 298.15 K and 310.15 K [3], were investigated by UV-Vis spectrophotometric measurements at the same ionic strength and T = 288.15 K, as well as at $0.50 \leq I$ / mol $L^{-1} \leq 1.00$ and T = 298.15 K. The speciation model obtained for the 3-hydroxy-4-pyridinone protonation was also confirmed by performing ^{1}H NMR titrations I = 0.15 mol L^{-1} in $NaCl_{(aq)}$ and T = 298.15 K.
The binding ability towards Ca^{2+} and Mg^{2+} was investigated using two analytical techniques, namely potentiometry (ISE-H^+) and UV-Vis spectrophotometry. The measurements were carried out at $0.15 \leq I$ / mol $L^{-1} \leq 1.00$ in $NaCl_{(aq)}$ and $288.15 \leq T$ / K ≤ 310.15.
The dependence on ionic strength of the thermodynamic parameters was modelled using an extended-type Debye-Hückel equation and the Specific Ion Interaction Theory (SIT), while the effect of temperature was studied by means of the Van't Hoff equation.
Furthermore, the sequestering ability of the ligand towards the metal cations under study was investigated by the determination, at different pH, ionic strength and temperature conditions, of the empirical parameter $pL_{0.5}$, already proposed by the research group. It represents the total concentration of ligand required to sequester the 50% of the metal cation present in trace in solution [4].

O3 SES1

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O4 SES1

SUPRAMOLECULAR AMPHIPHILES: FROM HOST-GUEST COMPLEXES TO CATANIONIC MIXTURES

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Amphiphiles have many applications in several fields, such as cosmetics and drug delivery etc. Thanks to their surface active properties these compounds are also named surfactants. Conventional surfactants consist of a hydrophobic alkyl chain and a hydrophilic head group, linked by covalent bonds. A new class of amphiphilic systems, the so-called supramolecular amphiphiles or supramphiphiles, has gained increasing attention in recent years. In this case the hydrophilic and hydrophobic components are held together by non-covalent interactions. Several strategies can be used to obtain supramolecular amphiphiles including host−guest, hydrogen bonding, charge-transfer, π-stacking, and Coulombic interactions [1,2]. Sulfonatocalix[n]arenes (SCn) are water-soluble macrocyclic host molecules formed by n 4-hydroxybenzenosulfonate units linked by methylene bridges in the *meta* position. With π-rich hydrophobic cavities and an upper rim decorated with negatively charged sulfonate groups, these hosts display high affinity and selectivity for positively charged organic species [3]. Besides simple host−guest complexes, SCn's were found to be very effective hosts for the construction of supramolecular amphiphiles owing to their special ability to induce the aggregation of suitable guest molecules. Self-assembled soft materials based on SCn are particularly attractive for biological/biomedical applications due to their low toxicity, water solubility and relatively simple synthesis yielding material on a multigram scale [4]. However, despite the interest in calixarene-based supramphiphiles, a quantitative characterization of the binding features and driving forces of the host-guest formation as well as the aggregation processes occurring in neutral aqueous solution has not been reported yet [5].
In this work, complex species, binding constants and forces driving the formation of supramphiphiles made of a p-sulfonatocalix[4]arene and positively charged long-tailed guests in neutral (buffered) aqueous solution have been determined by isothermal titration calorimetry (ITC) in order to find out the best systems and conditions for the assembly of efficient micellar-like aggregates. The aggregation features of the most promising host–guest complexes have been also studied by ITC in neutral aqueous solution. CMC and ΔHmic values of the micellar-like aggregates formed by different supramolecular surfactants highlight the crucial role played by the calixarene scaffold in the formation of efficient self-aggregating systems. Furthermore, mixed systems formed by the supramolecular amphiphiles and selected cationic surfactants have been studied.
The synergistic properties and the structural changes of the resulting intrinsic catanionic mixtures have been evaluated [6].

O4 SES1

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O5 SES1

SELECTIVE CHELETORS TO COPPER (II): A THERMODYNAMIC APPROACH

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Copper, iron and manganese ions act as cofactors for essential enzymes. Although absolutely essential for biological activity, excess redox-active metal ions have been associated with severe neuro-degenerative diseases [1–4]. Copper ions are essential for biological function, however are severely damaging when present in excess as catalyze the production of hydroxyl radicals that can irreversibly alter essential bio-molecules. Hence, selective copper chelators that can remove excess copper ions and alleviate oxidative stress will help assuage copper-overload diseases. Most currently available chelators are non-specific leading to multiple undesirable side-effects. The objective of this study was to verify the possibility of selectively chelate Cu(II) by using curcumin as ligand. Curcumin (HCur, 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a neutral yellow-orange colorant with a wide variety of pharmacological properties, such as anticancer and antitumor activities [5]. In addition, curcumin is thought to have beneficial effects in disorder of the neurological system including Alzheimer's disease [6].
Following our previous studies on the complexation behaviour of biological ligands towards some bioavailable metal cations, here we present an experimental investigation (potentiometric measurements, ^{1}H-NMR and UV-Vis spectroscopy) to obtain thermodynamic and structural properties in aqueous solution of the system whose general equilibrium can be written as follows:

$$p\,Cu^{2+} + q\,(OH)^{-} + r\,Cur^{-} \rightleftarrows Cu_p(OH)_q(Cur)_r^{(2p-q-r)} \qquad \beta_{pqr}$$

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O1 OMS1

A COMPREHENSIVE PEPTIDOMIC APPROACH TO CHARACTERIZE THE PROTEOMIC PROFILE OF SELECTED DURUM WHEAT GENOTYPES AND ITS IMPLICATION FOR COELIAC DISEASE AND WHEAT ALLERGY

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In the last decades, the varietal selection undertaken by breeders tailored to improve technological and productivity related traits, caused a considerable impoverishment of the genetic diversity of wheat varieties present on the market. Starting from this, the researchers are encouraged to investigate the natural diversity of available wheat genotypes in light of their potential to encode a lower number of celiac disease epitopes [1].
In a recent investigation we presented the detailed characterization of a tetraploid wheat collection containing 38 accessions of durum wheat (*Triticum turgidum*) selected from a wider list of 240 genotypes, developed at University of Bari Aldo Moro, including both wild and cultivated accessions [2]. The collection was investigated by a multidisciplinary approach including conventional proteomic profiling focused on the gliadin fraction (HPLC-UV and R5-ELISA), yield and quality traits of the whole grains [2]. A statistical evaluation of the acquired data set allowed the identification of a short list of candidate genotypes combining reduced gluten content with satisfactory rheological properties required for their perspective usability in bread or pasta [2].
As follow-up of that work, in the present communication an in-depth analysis of the proteomic profile of the selected wheat genotypes will be presented. Advanced proteomic approach was carried out combining proteins/peptides sequence information retrieved by specific enzymatic digestions (single and dual enzymes) with protein digestibility information provided by in-vitro simulated human gastroduodenal digestion experiments (see Figure 1 for details). The latter was applied to raw flours according to the standardized static protocol proposed by Minekus et al. in 2014 [3]. In both cases, the peptide pools were analysed by liquid chromatography high resolution tandem mass spectrometry in *data dependent*^{TM} acquisition mode. The instrumental method was customized in order to increase the amount of information retrieved and a dual-round software-based sequence identification with exclusion list was applied. The raw data were processed by the commercial software Proteome Discoverer 2.1 relying on the Sequest HT searching algorithm against a customized database containing all *Triticum* (Tax ID 4564) sequences available on UniProt DB.
The full list of enzyme specific peptides and gastroduodenal resistant peptides were filtered according to specific criteria of reliability for the highest identification confidence and finally, the refined list was screened for in-silico toxicity/immunogenicity risk assessment. Given the global information provided by the designed proteomic approach the risk assessment was

carried out not only tracing for potential toxicity for celiac disease patients, but also scouting for immunogenic sequences relevant for wheat allergic patients, achieving a comprehensive characterization of the selected genotypes. Various open-source bioinformatics tools were used for epitopes matching (*www.allergenonline.org/celiachome.shtml*, www.iedb.org).
The selected genotypes were assessed to encrypt a lower number of number of toxic/immunogenic epitopes for celiac disease and wheat allergy, and as such they could represent convenient bases for breeding practices and for the development of new detoxification strategies.

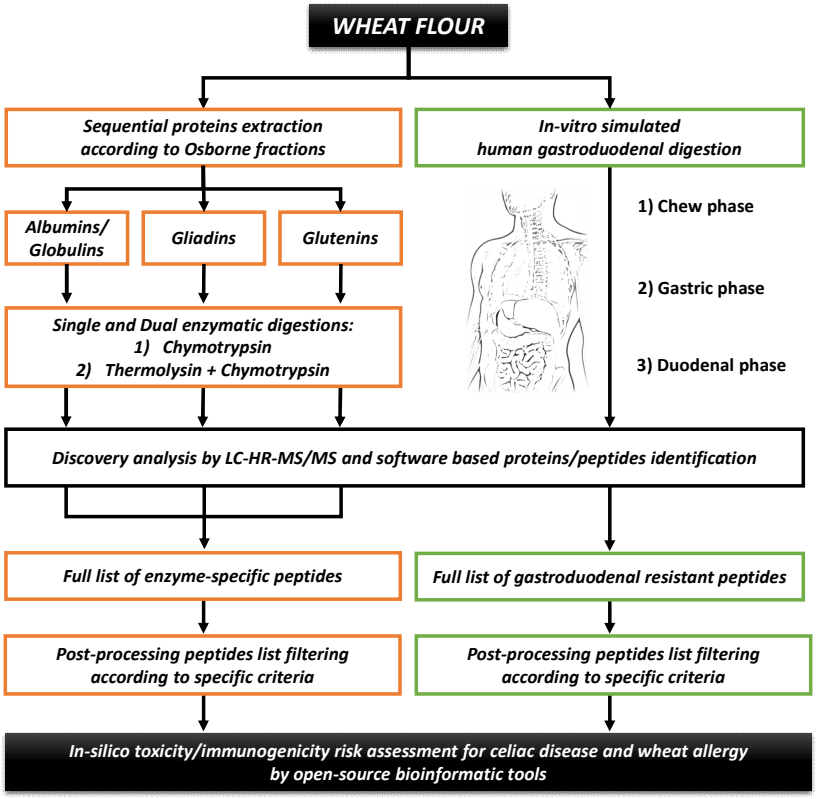

Figure 1. Scheme of the comprehensive peptidomic approach carried out to characterize systematically the proteomic profile of selected durum wheat genotypes.

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O2 OMS1

UHPLC-QqTOF UNTARGETED METABOLOMICS OF NATIVE PHENOLIC COMPOUNDS IN DIFFERENT RASPBERRY SAMPLES (*RUBUS IDAEUS L.* AND *RUBUS OCCIDENTALIS L.*)

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Dietary health benefits are increasingly associated to the consumption of phenolic antioxidants (e.g. anthocyanins and ellagitannins), which are widely distributed and abundant in small fruits, such as raspberry [1, 2]. The in-depth metabolomics profiling of polyphenol-rich fruits is of paramount importance for the discrimination of different berry species and/or cultivars. Moreover, an accurate knowledge of fruit native compounds is of crucial significance when their fate during the digestion processes is investigated [3, 4]. Accordingly, the aim of this work is to provide a detailed profile of these bioactive compounds in different raspberry species (*Rubus idaeus* L. and *Rubus occidentalis* L.) by untargeted approaches developed with UHPLC analysis coupled with quadrupole/time-of-flight mass spectrometry. Total soluble polyphenols (TSP), total monomeric anthocyanins (TMA), as well as targeted individual phenolic compounds were also determined in the extracts of the freeze-dried samples. The metabolomics profiling evidenced a high number of polyphenols mostly belonging to the anthocyanins, ellagitannins (ETs) and flavan-3-ols classes. TSP and TMA assays revealed a polyphenol content ranging from 198.5 to 337.2 mg procyanidin B1 eq. 100 g^{-1} f.w. and from 61.0 to1245.1 mg cyanidin-3-sophoroside eq. 100 g^{-1} f.w., respectively. Individual compound quantitation highlighted a general content of each selected analyte ranging from hundreds µg to tens mg 100 g^{-1} f.w.

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O3 OMS1

EICOSANOIDS AND ISOEICOSANOIDS ANALYSIS IN DRIED BLOOD SPOTS AND ORAL FLUID SAMPLES: A FASCINATING BIOANALYTICAL CHALLENGE

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Eicosanoids and isoeicosanoids are important signalling molecules derived respectively from the enzymatic and non-enzymatic oxidation of polyunsaturated fatty acids (PUFAs) [1]. The production of these lipid mediators is considerably increased during inflammation and oxidative stress, which play a key role in the pathogenesis and pathophysiology of a great number of diseases, such as neurological disorders, diabetes, renal dysfunction and cardiovascular diseases [2]. The analysis of these metabolites in minimally invasive biological specimens, e.g. oral fluid and dried blood spots (DBSs), can be extremely useful in elucidating their biological activity and potential biomarker role in the clinical setting. Their quantification represents a very challenging task due to the very low concentration levels (ppts range) and the tiny amount of sample available in the case of DBSs [3].
This work illustrates innovative procedures that combine micro-extraction by packed sorbent technique with ultra-high performance liquid chromatography coupled to electrospray ionization-tandem mass spectrometry for the determination of eicosanoids and isoeicosanoids in DBSs and oral fluid. The proposed analytical methods were fully validated and showed satisfactory precision (RSD ≤ 10%), recovery (90-110 %) and LODs in the range of 10-100 pg mL^{-1}. The straightforward application of the present methods for both the monitoring of preterm newborns suffering from Patent Ductus Arteriosus and Heart Failure patients is widely displayed, highlighting the importance of eicosanoids and isoeicosanoids in leading disease progression and responsiveness to the therapy.

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O4 OMS1

INVESTIGATING THE PROTEOMIC PROFILE OF HT-29 COLON CANCER CELLS AFTER *LACTOBACILLUS KEFIRI* SGL 13 EXPOSURE USING THE SWATH METHOD

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Despite some studies revealed that kefir acts on different cancers such as colorectal cancer [1], the proteomic changes that occur in the colon cancer cells remain to be explored.
In this study, the proteomic analysis was combined with determination of kefir characteristics (e.g. adhesion capacity, gastrointestinal and antibiotics resistances), in order to confirm its use as a probiotic. Therefore, a label free strategy based on SWATH-MS was applied to investigate the proteomic profile of HT-29 cells after exposure for 24 hours to a specific strain of *Lactobacillus kefiri* named SGL 13. We identified a total of 60 differentially expressed proteins in HT-29 cells, among which most are located into the extracellular exosome, playing important/crucial roles in translation and cell adhesion, as indicated by the enrichment analysis. The eIF2 and retinoid X receptor activation pathways appeared to be correlated with the anti-tumoral effect of SGL 13. Immunoblot analysis showed an increase in Bax, and a decrease in caspase 3 and mutant p53, and ELISA assay revealed inhibition of IL-8 secretion from HT-29 cells stimulated with LPS upon SGL 13 treatment, suggesting pro-apoptotic and anti-inflammatory properties of kefir.
In conclusion the results of this study, the first of its kind using co-culture of kefir and colon cancer cells, demonstrated that *L. kefiri* SGL 13 possesses probiotic potency and contribute to elucidate the molecular mechanisms involved in the *L. kefiri*-colon cancer cell interactions. This study represents the first ever analysis based on SWATH for the molecular characterization of the effects induced by kefir on colon cancer cells. The results obtain are relevant also by a technical point of view because they show the potentialities of an investigation done by SWATH-MS also to study the effects of probiotics.

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O5 OMS1

ADVANCED LC-MS-BASED APPROACHES FOR THE INVESTIGATION OF PEPTIDES AND PROTEINS

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In proteomic analysis, scientists are more and more challenged in implementing separation systems capable to provide enhanced separation power, as well as specificity/sensitivity of detection for adequate identification/quantification of the separated compounds. Gel-free, LC based separation techniques come along with the benefits of higher throughput, relative speed, capability of quantitation, and easiness of full automation. From the detection standpoint, the advent of ESI and MALDI ionization techniques have definitely concurred to make LC–MS and LC–MS/MS emerge to a central role in modern proteomics. Furthermore, several two-dimensional comprehensive LC platforms (LCxLC) have been successful in delivering an exponential increase in terms of separation efficiency, the latter in turn allowing for more reliable identification of low-level sample constituents and potential biomarkers.
This presentation will focus on different approaches for high resolution front-end separation of intact and digested proteins, relying on the use of RP-LC due to its amenability of direct linkage to MS. Moreover, in LCxLC approaches the use of RP-LC in both the dimensions alleviated major technical challenges, and allowed for greater flexibility in the choice of column dimensions and operation modes. High efficiency was achieved through the use of fused-core (2.7 μm d.p.) stationary phases (4.6 or 2.1 mm I.D. columns), and the selectivity adjusted by careful selection of the mobile phase pH (basic or acidic buffered solution), and column temperature (35-60 °C). In contrast to shotgun proteomic approaches, the complexity of the ions entering the MS is reduced, avoiding to overwhelm the limited dynamic range per spectrum of the mass detector.

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O1 FN3

GRAPHENE–BASED ELECTROCHEMICAL SENSOR FOR THE DETERMINATION OF VITAMIN C IN FOOD AND FORMULAE FOR INFANTS AND YOUNG CHILDREN

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A simple and rapid voltammetric method based on disposable carbon screen-printed electrodes modified by a novel hybrid nanocomposite, formed of 1-pyrene carboxylic acid functionalized reduced graphene oxide flakes, surface decorated by organic-coated Au nanoparticles (Au/RGO/SPCE) is proposed for the determination of vitamin C. Vitamin C is a water-soluble vitamin that refers to L-ascorbic acid (L-AA) and compounds exhibiting biochemical activity equivalent to L-AA, namely its oxidation product (dehydroascorbic acid). Vitamin C needs to be provided by the diet since humans can not synthetize it. Vitamin C is added to foods and formulae, especially for infants and young children, and it is crucial that manufactures meet the required values to ensure an equilibrate, safe and adequate diet. Different methods have been proposed for vitamin C determination and monitoring. Among these, HPLC-based analysis coupled to spectrophotometric detection has the advantage to be a reliable, robust reference method. Enzymatic methods using commercial test kits are also frequently used in control laboratories. The disadvantages are the associated high costs. The development of rapid, cost-effective screening analytical methods is recommended for a massive monitoring program during foodstuff production and storage.
The aim of this study was to develop a rapid, cost effective and reliable electrochemical sensor for vitamin C quantification (L-AA) in milk-based samples. Estimation of the linear range, calibration function, limit of detection (0.5 μM) and reproducibility was performed. The proposed analytical system was successfully applied for the determination of vitamin C in commercially available food and formulae for infants and young children.

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O2 FN3

THE PRICKLY PEAR PEEL (OPUNTIA FICUS-INDICA (L.) MILL.): AN EXAMPLE OF SOURCE OF A BIOACTIVE COMPOUNDS OBTAINED USING AN ECO-INNOVATIVE EXTRACTION TECHNOLOGY, EXTRACTOR NAVIGLIO®

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The Prickly Pear (Opuntia ficus-indica L. Mill.) Belongs to the Cactaceae family and grows in different parts of the world such as: in North America (Mexico and the United States), in South America (Argentina, Peru, Bolivia and Brazil), in Africa (Morocco, Tunisia, Eritrea, Ethiopia and South Africa), in Europe and Asia (Spain, Italy, Israel and Iran). Among these, Mexico is the world's largest producer of prickly pear (more than 400,000 tons/year)[1]. The prickly pear shows an enormous genetic variability being a polyploid, in particular octoploid; this variability could reflect the diversity of the colors of the prickly pear fruit that varies between red, purple, green, orange and yellow[2]. Prickly pear has a high quantity of peel (between 40-45% of the total weight of the fruit), which generally represents the discarded fruit processing. However, this by-product can be a good source of bioactive compounds[3] that could be obtained using different technologies and eco-innovative extraction techniques such as the Extractor Naviglio®[4]. This is one of the techniques that has itself best to solid-liquid extraction in the field of bioactive molecules and compounds. In fact, from the peel natural pigments can be extracted which, in modern industry, have attracted the attention of both producers and consumers thanks to their proven safety with respect to synthetic dyes. Natural pigments are generally compounds that promote beneficial health effects, positively influencing biological activities[5] due to their antioxidant potential, for anti-inflammatory, antidiabetic, antitumor and antimicrobial activity, showing preventive effects against various diseases such as cancer, neurodegenerative and cardiovascular diseases, among others[6]. The color stability and antioxidant activity of these pigments, however, are limited due to their rapid degradation in the presence of factors such as oxygen, light, pH or temperature[7]. The peel of prickly pears is rich in betalain which are generally classified into two groups, betacyanins and betaxantines, based on their structural characteristics and light-absorbing properties. Both are water-soluble pigments: the betacyanins give the red-violet color and the betaxantines confer the yellow-orange color[8]. All betalain are based on a common structural unit, betalamic acid (Figure 1), which condenses with various amino acids or groups of free amines, or structures containing indoline to form betaxanthins or betacyanins respectively[9]. The present study deals with the evaluation of the application, for the first time, of the dynamic solid-liquid extraction method of the Extractor Naviglio®, of the effects of the pH (pH≤5.0), of the extraction solvent

(water mixture: ethanol in 80:20 ratio) and storage conditions (environment and refrigeration) on the content of betacyanin and betaxantine in prickly pear skin extracts. It was noted that factors such as pH, storage time and temperature influenced color stability, according to literature data[10]. Water extraction and the use of lower temperatures (≈4°C) could be applied to extract a more interesting quantity of betacyanins and betaxantines from prickly pear skin. A good recovery of these colors of prickly pear peel, which today is a waste product of the food industry, could allow an interesting use as an alternative to synthetic dyes.

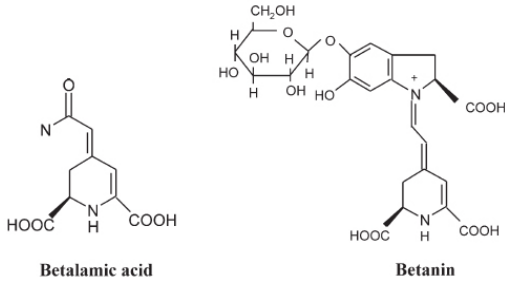

Figure 1. Structures of betalamic acid and betanin.

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O3 FN3

NEW VALUABLE RESOURCES FROM AGROINDUSTRIAL BY-PRODUCTS FOR A MULTITUDE OF APPLICATIONS IN PACKAGING AND BIOTECHNOLOGY FIELDS

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Recent directives of the European Community are implementing the ambitious Circular Economy plan, aimed at reducing waste and enhance recycling processes, with the main objective to "close the loop" of the productive cycle [1].
In this context, supporting the environmental sustainability, the use of by-products as new source represents an innovative trend, and is the object of many researches. In the agro-industrial field, high amounts of vegetables are discarded, sometimes just because they are not considered suitable for commercial use, for their dimension, shape or ripeness degree. Besides, in industrial preparation of canned and under-oil vegetables, such as onion or artichokes, large portions of the plant are eliminated, and farms also have to afford the costs for their disposal, with high environmental impact.
Those by-products are usually still rich of nutritional valuable substances; therefore, to introduce them again in the productive cycle as new resources can provide an additional economic value.
The aim of this study is the re-evaluation of by-products from onions, artichokes, asparagus, cardoons and grapes, proposed as source to prepare extracts with different solvents, and by different procedures, such as the use of microwave assisted extraction.
The presence of several valuable bioactive substances was identified in all considered materials using several analytical techniques. The amount of phenolic compounds was evaluated by spectrophotometric assay, following the Folin-Ciocalteu method. Reversed phase chromatography equipped with UV/DAD and mass spectrometry was used for the identification of the main compounds. A relevant antioxidant power was evaluated by Oxitest, a reactor able to measure the oxidative stability of vegetable oil enriched with the extracts. Steric exclusion chromatography was used to separate fractions according to their different molecular weight. Molecules with prebiotic activity, such as fructooligosaccharides and inulins were found in extracts from residues of cardoons, onions and artichokes by high performance anionic exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD).
The potential applications of the extracts obtained can cover a multitude of sectors, comprehending the nutraceutical, cosmetic, herbalist, food technology, and packaging fields. Functional foods can be obtained by adding extracts to food products; the peculiar technological features of inulins to form gel when mixed with water, can be exploited to

obtain bulk effect that improves food texture of creamy products. Besides, an important synergic effect is reported by the co-presence of phenolic compounds with prebiotics, linked to the increase of bioavailability due to the glucosidases released by bacterial enzymes [2].
As for packaging, innovative material can be produced from these substrates, representing a solution for new technologies, as requested by the new European Directive that banned the single-use plastics by 2021. Active and compostable packaging based on biopolymers obtained from natural ingredients can be realized using only edible substances as reagents [3]. The materials can be enriched with natural and active molecules having antibacterial and antioxidant properties, thus exerting a protective effect on the packaged food. The application of the active film on products such as meat, fruits and vegetables showed its efficacy in prolonging their shelf-life. Finally, the solid residues of extraction, mainly constituted by cellulose and lignine, could be used as raw material for obtaining paper to be adopted as secondary packaging.

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O1 SES2

STUDY ON THE COORDINATION CAPABILITY OF KOJIC ACID DERIVATIVES TOWARDS OXOVANADIUM(IV)

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Oxovanadium(IV) complexes exhibit insulin-mimetic activity and may be good candidates for the treatment of type II diabetes mellitus [1]. In particular, the complexes of maltol, in which the cation is coordinated by two 3-hydroxy-4-pyrone units, have undergone extensive pre-clinical testing [2].
Kojic acid (5-hydroxy-2-(hydroxymethyl)-4-pyrone, KA) shows a hydroxyl-pyronic structure and the coordination of KA, or its derivatives, with oxovanadium(IV) was previously studied. Yuen et al. [3] compared the glucose-lowering properties of BMOV (bis(maltolato)oxovanadium(IV)) and bis(kojiate)oxovanadium(IV) complexes, Sanna et al. [4] studied the chemical equilibria of KA-vanadyl complexes in blood serum and Wei et al. [5, 6] developed and tested a series of complexes based on the KA structure for the glucose control in blood. These studies showed a glucose-lowering activity of bis(kojiate)oxovanadium(IV) complexes.
In this work the complexation capability of four KA derivatives towards oxovanadium(IV) was studied. The newly synthetized ligands (namely S2, S3, S4 and SC, Figure 1) have two or three kojic acid units linked through diamines or tris(2-aminoethyl)amine chains, respectively. The synthesis and the characterization of the four ligands considered were previously presented, as well as their coordination capability towards Fe^{3+}, Al^{3+}, Cu^{2+} and Zn^{2+} cations [7, 8].
The chemical systems were studied by potentiometry and UV-visible spectrophotometry at 25°C and ionic strength 0.1 mol L^{-1}, KCl. EPR spectra were recorded both at room (RT) and low (LT) temperature, as a function of pH. For all systems a chemical model was hypothesized analyzing the experimental data by a thermodynamic approach and by chemometric methods.
The formation constants of the complexes and the pure UV-vis and EPR spectra were determined.
In all systems the coordination of the oxovanadium(IV) starts already under acidic conditions and the metal complex remains stable even at pH 8. Ligands S2, S3 and S4 form two complex species with two kojate units inserted in the coordination shell successively.

O1 SES2

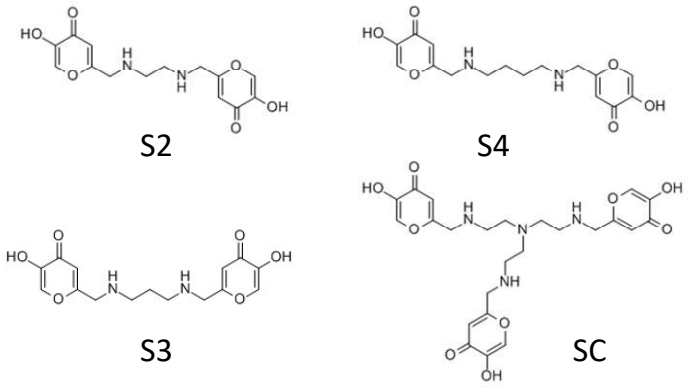

Figure 1. Molecular structure of the kojic acid derivatives studied: S2: [ethane-1,2-diylbis(iminomethanediyl)]bis(5-hydroxy-4H-pyran-4-one), S3: [propane-1,3-diylbis(iminomethanediyl)]bis(5-hydroxy-4H-pyran-4-one); S4: [butane-1,4-diylbis(iminomethanediyl)]bis(5-hydroxy-4H-pyran-4-one); SC: 6,6′,6″-(((nitrilotris(ethane-2,1-diyl))tris(azanediyl))tris(methylene))tris(3-hydroxy-4H-pyran-4-one).

The shifts of the UV-vis absorption bands as well as of the magnetic parameters reveal that four ligand oxygen atoms replace the water molecules in the equatorial coordination plane leading to the formation of a dominant complex species with stoichiometry [$VOLH_2$] in the 4-8 pH range. Despite the presence of a third KA unit in the SC ligand, only two of them participate to the coordination process.
The sequestering capability of the four ligands towards oxovanadium(IV) is very similar and it is higher than that of maltol and KA.

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O2 SES2

NATURAL AND SYNTHETIC POLYMERS: CHARACTERIZATION OF ACID-BASE BEHAVIOUR AND BINDING PROPERTIES

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Natural and synthetic polymers are employed in different fields, as thickening, dispersing, suspending, and emulsifying agents in pharmaceuticals, cosmetics, paints, industrial formulations and in medical applications such as gels for skin care or skin disease treatment products. The polymers are inexpensive, safe and available in a variety of structures with different characteristics. A wide number of derivatizable groups and of molecular weights, varying chemical composition of these polymers also provide opportunities in drug delivery of therapeutic agents. The polyelectrolytes reported in this study are: polyethylene glycole (PEG), Acusol 445 (homopolymer of acrylic acid), carboxymethylcellulose (CMC) and Carrageenan. The characterization of the acid-base behaviour of the different polymers were studied in $NaNO_3$ aqueous solutions, at I = 0.15 mol dm^{-3} and T = 298.15 K. In the calculation of the protonation constants, a simplified approach was used, treating a polyelectrolyte like a simple low molecular weight ligand defining a minimum number of protonation sites necessary to extensively describe the system. The binding ability of the polymers towards two different metal cations, Zn^{2+} and Sn^{2+} was studied in $NaNO_3$ aqueous solutions at I = 0.15 mol dm^{-3} and T = 298.15 K. The speciation models of the different systems were compared among them and sequestering ability of these polyelectrolytes was evaluated by means the empirical parameter pL_{50}.

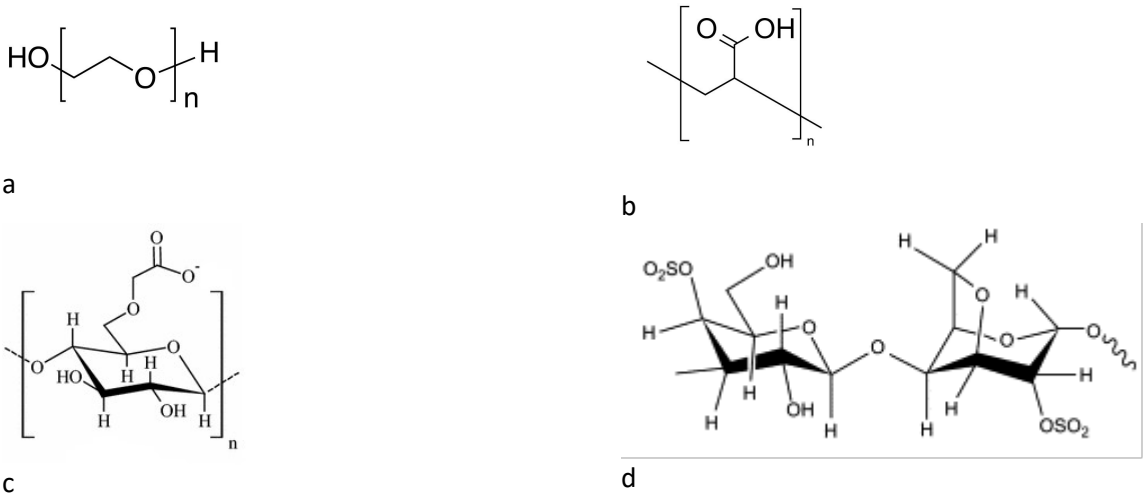

Figure 1. Structure of the polymers: a PEG, b Acusol 445, c CMC, d Carrageenan.

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O3 SES2

INTERACTION OF BERYLLIUM (II) ION WITH GLUTATHIONE IN AQUEOUS SOLUTION

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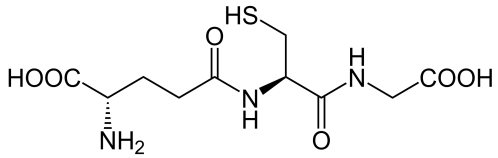
Beryllium is found naturally into various forms like gemstones, beryl and chrysoberyl. It is also used in the electronics industry as high thermal conductivity material and in nuclear industry for its high neutron moderating ability [1]. Chronic beryllium disease (CBD) is an occupational lung disorder, in exposed industry workers. Recent studies have identified a strong association between CBD and some human leukocyte antigen that contain glutamic acid [2].



Glutathione (γ–L–glutamyl–L–cysteinyl–glycine)

The complexation between Be^{2+} ion and glutathione (H_3L) has been studied at 25° C in 0.1 mol dm^{-3} $NaClO_4$ as ionic media, by potentiometric measurements of the hydrogen ion concentration.

The determination of the equilibrium constants has been carried out by evaluating the quantity Z_H, which represents the average number of protons released per glutathione molecule, as a function of the pH. Experimental data for Be^{2+}–glutathione system, processed by *Hyperquad* program [3], are interpreted by the following equilibria:

$$Be^{2+} + H_3L = BeH_{-2}(H_3L) + 2\ H^+ \qquad \log(\text{const.}) = -3.19 \pm 0.04$$

$$Be^{2+} + H_3L = BeH_{-4}(H_3L) + 4\ H^+ \qquad \log(\text{const.}) = -11.71 \pm 0.06$$

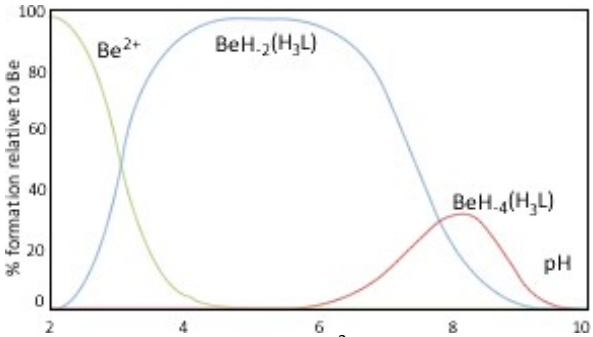
Furthermore, the interaction between Be^{2+} and glutamic acid (H_2A), conducted in the same experimental conditions, has been also considered to understand the role of glutamic acid residues toward the complexation. Measurements are consistent with equilibrium:

$$Be^{2+} + H_2A = BeH_{-2}(H_2A) + 2\ H^+ \qquad \log(\text{const.}) = -6.30 \pm 0.08$$

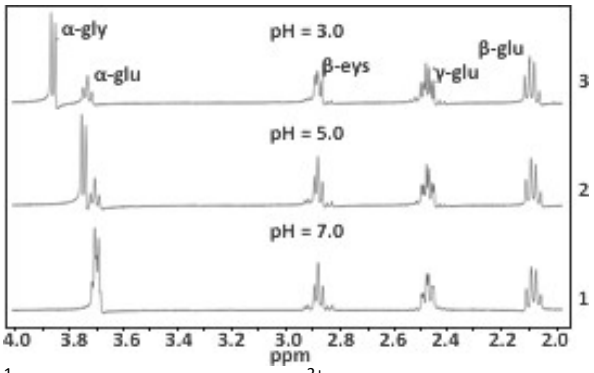
To establish which sites of the ligand are involved in the coordination with the berillyum ion, ^{1}H–NMR spectra are obtained in absence and in presence of metal ion as a function of pH.

O3 SES2

From spectra, recorded at different pH value, is evidence that only glutamic residue is involved in complexation.



Distribution diagram for Be^{2+}–glutathione system ($1.0 \cdot 10^{-3}$ M Be^{2+}, $2.0 \cdot 10^{-3}$ M Glutathione, 0.1 M $NaClO_4$).



^1H–NMR spectra in Be^{2+}–glutathione solutions ($1.0 \cdot 10^{-3}$ M Be^{2+}, $2.0 \cdot 10^{-3}$ M Glutathione, 0.1 M $NaClO_4$, 10% D_2O), at different pH value.

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O1 OMS2

WATER-ASSISTED TRAP FOCUSING FOR ULTRA-LARGE VOLUME INJECTION IN REVERSED-PHASE NANO-LIQUID CHROMATOGRAPHY-ELECTRON IONIZATION MASS-SPECTROMETRY

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Nano HPLC gradient elution is the separation method of choice in many emerging LC-MS applications. This success is due to the synergistic effect of nano HPLC flow rates and ionization efficiency. However, the good mass sensitivity of nano HPLC is diminished by the severe injection volume limitation. Solvent-based solute focusing of aqueous samples in short trap columns, operating in switching mode, can overcome this constraint. Nevertheless, if the injection volume is too large or when the sample is in organic solvents, solutes are poorly retained by the trap during injection, and volume overload can occur, leading to altered peak shapes and signal loss. We present an efficient, instrumental method which relies on water dilution to assist trap solute focusing. An Agilent 1290 Infinity II UHPLC pump was used to deliver 20 μL/min of water to assist the dilution and trap focusing of a 20 μL sample in organic solvent (CH_3OH or CH_3CN). Trap elution (Agilent AdvanceBio-Mapping trap column 0.3 x 5 mm x 2.7 μm) and chromatographic separation (Agilent Zorbax SB-C18 0.0.75 x 150 x 3.5 μm) were carried out with an Agilent 1100 series nano pump at a flow rate of 400 nL/min. Gradient elution was from 100% of solvent A (97% water:3% ACN, v/v) to 100% of solvent B (ACN) in 10 min. An Agilent 7010B QQQ mass detector was equipped with a LEI LC-MS interface set at 400°C. MS data were acquired in MRM and SCAN modes. A 100% water flow delivered by the pump was directed to a first tee-union (T1) (Figure 1) where it was split into two stream channels. At 20 μL/min, the selected split ratio generated two streams: (A) 16.5 μL/min and (B) 3.5 μL/min. The higher flow stream was directed to a second tee-union (T2), while the lower one passed through a six-port valve (V1) before reaching T2. The valve was equipped with a 20 μL sample loop. A sample, in organic solvent, was injected into the loop. Trap loading: V1 was switched and the sample was carried at 3.5 μL/min to T2 mixing tee. The two streams mixed inside T2 carrying the sample at 20 μL/min into the trap in an aqueous environment for an optimized sample focusing. The trap was connected to a second six-port valve (V2) for back-flushing operation. To evaluate the T2 dilution at different distances from the T-junction exit, the transport phenomena equations, which express the conservation of mass, and momentum of chemical species were

numerically solved within the COMSOL Multiphysics® environment. Under the hypothesis of isothermal, incompressible Newtonian fluids and laminar mixing flow, preliminary investigations allowed quantifying the influence of the parameters that affect the flow behavior. Moreover, due to the laminar nature of the flow rate, a microfluidic chip (Dolomite Inc, Royston, UK) was used instead of T2 to improve mixing of the two solvents when in trapping mode.

Once the sample was fully transferred and loaded into the trap, V2 was switched allowing the trap to be back-flushed by the nano HPLC solvent gradient at 400 nL/min in 10 min.

To demonstrate the feasibility of this approach, pesticide mixtures in organic solvent extract from a soil matrix was considered. Good results in term of peak width and chromatographic resolution were obtained. Limit of detections of 10 and 100 μg/Kg in MRM and full scan modes were achieved, respectively.

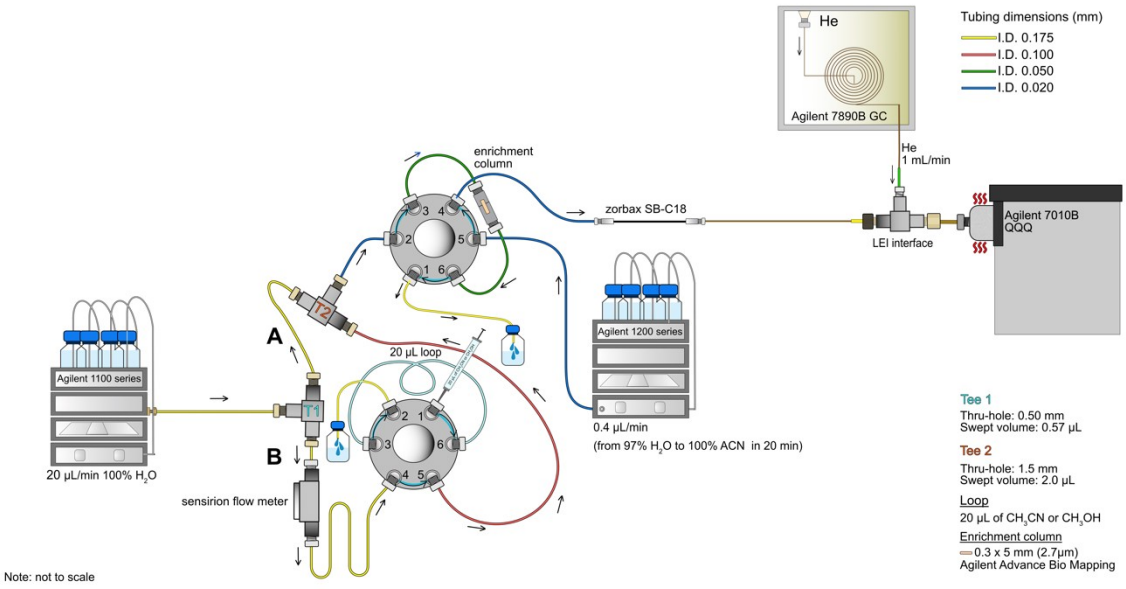

Figure 1.Overview of water assisted trap focusing system in loop loading position. A: higher flow rate; B: lower flow rate.

O2 OMS2

REIMS, A NOVEL AMBIENT MASS SPECTROMETRY METHOD FOR THE REAL-TIME IDENTIFICATION OF FOOD SAMPLES

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Rapid Evaporative Ionization Mass Spectrometry (REIMS) is a novel ambient MS method that can be used for the real-time identification of unknown samples, without any sample pretreatment. Such an approach is based on the analysis of the vapor produced by thermal ablation. MS profile represents an univocal fingerprinting, usable for geography evaluation and authenticity assessment of foodstuffs. For this purpose, a database of authentic samples need to be created.
The REIMS method was employed for the fast characterization of different food products (fish, cheese, olive oil). It operates using an electrosurgical knife (iknife), which creates an aerosol that is evacuated from the sample through a transfer line into the ionization source, where a heated collision surface is located for the thermal ionization process. A multivariate statistical algorithm was validated for the real-time identification process.
High value products, granted with PDO (Protected Designation of Origin) indication, were analyzed and successfully differentiated, without any geographical mismatching, thus demonstrating the applicability of the new technique in the detection of food fraud. The new technology was also applied to commercially popular and genetically similar Mediterranean Sea fish to obtain fast and accurate speciation results.
Iknife represents a powerful tool in the preservation of food security and safety. It can be also used as a shotgun approach to achieve a comprehensive characterization of a complex sample and, since lipids are normally the most representative detected molecules, it can be successfully employed in lipidomics.

O3 OMS2

UNTARGETED LC-HRMS^n VERSUS TARGETED LC-MS/MS AND PRE-COX-LC-FLD FOR DETERMINATION OF PARALYTIC SHELLFISH POISONING TOXINS AND TETRODOTOXIN IN SEAWATER AND SHELLFISH

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Paralytic shellfish poisoning toxins (PST) are a group of neurotoxins produced by marine dinoflagellate belonging to genus *Alexandrium*, *Gymnodinium* and *Pyrodinium* as well as by freshwater cyanobacteria that may contaminate drinking water supply. PST represent a major concern for humans since a fatal neurological syndrome may occur following ingestion of contaminated seafood. Tetrodotoxin (TTX) is also a naturally-occurring toxin produced by marine bacteria, well known in Japan to cause lethal food poisonings following ingestion of contaminated puffer fish (*fugu*).

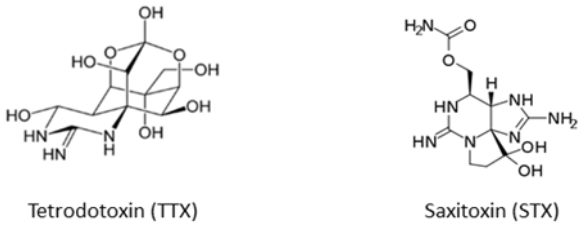

Figure 1. Structures of tetrodotoxin (TTX) and saxitoxin (STX), the parent compound of the PST group of toxins.

Despite presenting different structural features, PST and TTX exert similar toxic effects and, most importantly in an analytical perspective, they are co-extracted under the same conditions; thus availability of a methodological approach for their combined detection is desirable. Whilst PST are regulated and officially monitored in Europe, more data on TTX occurrence in bivalves and gastropods are needed before meaningful regulations can be established.

In this study [1], we used three different analytical methods - based on i) hydrophilic interaction liquid chromatography with high resolution multiple stage mass spectrometry (HILIC-HRMS^n), ii) ultrahigh performance HILIC-MS/MS, and iii) pre-column oxidation with liquid chromatography and fluorescence detection (Pre-COX-LC-FLD) - to investigate the presence of PST and TTX in seawater and shellfish (mussels, clams) collected in spring/summer 2015 to 2017 in the Mediterranean Sea. Samples were collected at 10 sites

in the Syracuse Bay (Sicily, Italy) in concomitance with a mixed bloom of *A. minutum* and *A. pacificum*. A very high PST contamination in mussels emerged, unprecedentedly found in Italy, with maximum total concentration of 10850 μg STX eq/kg of shellfish tissue measured in 2016. In addition, for the first time TTX was detected in Italy in most of the analyzed samples in the range 0.8-6.4 μg TTX eq/kg.

The recurring blooms of PST-producing species over the 3-year period, the high PST levels and the first finding of TTX in mussels from the Syracuse bay suggest that human health concerns exist and that monitoring programs of PST and TTX in seafood should be activated in this geographical area. Three different instrumental platforms and 3 separate analytical methods were used for analysis of these samples. Each of these may be applicable to the high throughput testing of shellfish tissues in a monitoring framework, although currently Pre-COX-LC-FLD method is the only analytical method allowable within the EU law. Whilst the qualitative results compared well between the three approaches, some significant differences emerged, particularly in comparison with the HILIC-$HRMS^n$ approach. Further work is on-going to understand the reasons for such differences and to conduct formal validation of the HILIC-$HRMS^n$ method before the platform can be used routinely.

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O1 SB3

DEVELOPMENT OF CHEMILUMINESCENT SPLIT G-QUADRUPLEX BIOSENSOR FOR ANTIBODY DETECTION

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One of the principal findings of molecular and cellular biology is that cells metabolism and homeostasis are based on networks of interacting proteins, which regulation is often based on noncovalent interactions.[1] Inspired by this mechanism, we developed a proximity based system that take advantage of the antibody-activated assembly of a split DNAzyme G-quadruplex. To this end, DNAzyme split single strands, each functionalized at one end with antigen molecules, have been designed. Their binding to target antibody leads the co-localization of the two DNAzyme split single strands, with a consequent increase of their local concentration. This drives the two split G-quadruplex fragments self-assembly, followed by hemin binding to produce the active G-quadruplex DNAzyme structure, able to catalyze the luminol/H_2O_2 chemiluminescent reaction. We have demonstrated that our approach could be used for different antigen/antibody systems showing high binding affinity, specificity for the target antibody, and selectivity to work. This study highlights the potential of bio supramolecular DNA engineering for the development of innovative rapid bioanalytical assays, aimed at detecting specific antibodies in biological samples for diagnostic purposes.

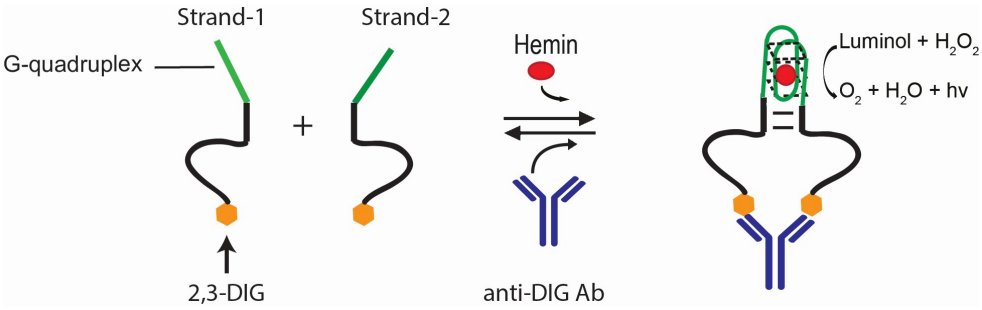

Figure 1. Antibody-templated assembly of the G-quadruplex DNAzyme. In this strategy, DNA G-quadruplex is split into two halves by the ratio of either 4: 8 (green in the figure), and each of the two strands is conjugated with a recognition element (antigen) specific for a target antibody. Only in the presence of the antibody the two fragments are colocalized in a confined space and can reassemble into the functional G-quadruplex DNAzyme structure which, in presence of hemin and luminol, provides a chemiluminescent signal.

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O2 SB3

A PRINTED POTENTIOMETRIC WEARABLE SENSOR FOR pH MONITORING IN SWEAT

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Nowadays, one of the hot topics in medical and bioanalytical field is the development of continuous, non-invasive, and easy to use sensing devices for biomarkers monitoring. Highly improved by the use of high technologies, wearable sensors have gained a primary role in the continuous and ubiquitous monitoring of people health state. Exploiting flexible electronics, smart materials and low-power working devices, wearable sensors can be easy produced, thanks to reduced barriers to technology accessibility and cost decreasing, unleashing the potential for simplified healthcare procedures. So that, sport activities monitoring and healthcare can be easily achieved by the end-user, allowing for the delivering of a detection platform at any time and any location, with positive implications for the monitoring of vital biomarkers during a physical performance as well as the treatment of chronic disease conditions. In order to create such user-friendly wearable devices, automatic and passive system have been fabricated, exploiting wireless technologies (i.e. Near Field Communication (NFC) or Bluetooth Low Energy (BLE)) for data transmitting, electrochemical techniques (i.e. potentiometry and amperometry) for analyte detection and printed electronic circuits for components communication [1,2]. It is clear that, the several components of the whole sensing platform, namely an efficient data collector and transmitting system, a small power supply (if necessary) and a miniaturized sensor, must be embedded in the same flexible substrate (i.e. PET), to create a small-size wearable sensor applicable on the body, namely the skin surface.
Herein, we developed a miniaturized wearable sensor for pH sweat real-time detection during a physical activity (Figure 1). The device consists of a low-cost fabricated screen-printed electrode (SPE), for pH detection by potentiometric measurements, closely integrated in a flexible Radio frequency identification (RFID) antenna working in the UHF band (868-960 MHz), for data transferring to an external receiver. Moreover, an electronic chip enabling the data storage, signal elaboration and wireless communication to a remote reader. Both the electronic and analytical components were embedded on a flexible Kapton substrate allowing for the fabrication of a small-sized and flexible sensor, applicable on epidermal surface.
In order to create a sensitive layer to the H^+ concentration in sample, the surface of the SPE was modified with a metal oxide compound, by electrodeposition of a solution containing iridium oxide. First, several parameters for the sensor optimization were studied, namely

number of scans and potential window for the metal electrodeposition, interferences study and pretreatment procedure for stability enhancement. After, a calibration curve was obtained by potentiometry measurements of standard solutions, with a linear response in the range between pH 4 and pH 8 and a regression equation equal to y = -0.069 x + 0.72, R^2= 0.989. Finally, pH was detected in real sample, i.e. sweat sample, obtaining a value of 5.2, in agreement with normal sweat values, equals to 4.5-6.

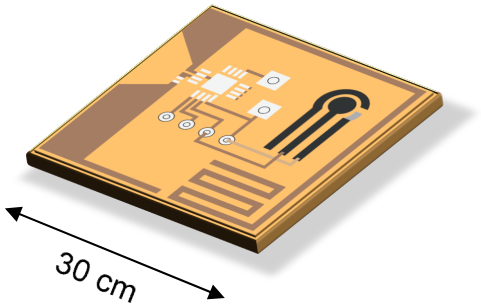

Figure 1. Picture of the developed wearable sensor

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O3 SB3

GET INSPIRED BY NATURE WITH BIOLUMINESCENT BIOSENSORS: "*PRENDERE LUCCIOLE PER LANTERNE*"

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Irrespective of the different molecular mechanisms, all chemical reactions catalysed by bioluminescent enzymes require molecular oxygen. An intriguing hypothesis suggests that bioluminescence arose in the early evolution of life because of its ability to remove oxygen, which was toxic to life when it first appeared on earth. Then, when oxygen became abundant, other more efficient antioxidant mechanisms evolved for oxygen removal, and most luminous species became extinct. Today, still many different bioluminescent species exist in nature, such as fireflies, bacteria, mushrooms, invertebrates, as well as fish.
Thanks to advancements in synthetic biology, organic chemistry and computational models, bioluminescence is widely exploited in several fields, ranging from the detection of microbial contamination to in vivo imaging to track cancer and stem cells, from cell-based assays for drug discovery to optogenetics to understand circuitry in the brain.
Here we report the generation of a portfolio of biosensors based on living cells and cell-free systems. We developed bioluminescence smartphone-based biosensing platforms exploiting highly sensitive luciferases as reporters in bacteria, yeast, and human cells lines. These biosensors, relying on reporter gene technology and split complementation strategies were integrated into 3D-printed cartridges. Smartphone-based cell biosensor were developed and applied to the detection of compounds with estrogenic, androgenic, and pro/anti-inflammatory activities. The comparison of analytical performance of whole-cell biosensors with the corresponding cell-free transcription/translation systems, which include the biological machinery and energy source to express a reporter protein as consequence of target activation, is also discussed.
Cell-free systems relying on "nano-lanterns" were developed to provide a ready-to-use and stable ATP sensing paper that can be easily integrated in miniaturized devices with smartphone detection. The feasibility of origami paper-based enzyme biosensors for detection of neurotoxic compounds is also reported exploiting both bioluminescence and its sibling, chemiluminescence, as detection principle.
Proof-of-concept applications of these biosensors are presented together with main limitations, such as those related to sensitivity and robustness, and current unsolved challenges to turn them into marketable biosensors.

O1 BIO2

SALIVA AS A TOOL FOR MONITORING OXIDATIVE STRESS IN SWIMMERS ATHLETES PERFORMING A VO2 CYCLE ERGOMETER TEST

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Physical exercise is considered one of the most important beneficial factors of a healthy lifestyle, able to minimize the risk of several disorders, such as cardiovascular and endocrine. Increasing number of scientific evidence indicates that prolonged and strenuous physical exercise can cause muscle fatigue, inflammation and oxidative stress, limiting the physical performance. Furthermore, chronic exposure to high levels of reactive oxygen species (free radicals), due to the unbalance between the pro-oxidant factors and antioxidant defenses, can become toxic and cause cell and tissue damage. Carbonyls and isoprostanes are the main compounds produced during oxidative stress, whereas uric acid is one of the most powerful salivary antioxidant.

The use of saliva in the monitoring of physical exercise is an attractive approach because this technique is less invasive and safer. Saliva samples may be collected several times from one subject, allowing a sort of real-time monitoring during and after physical tests, training or competitions. Thanks to the rapid equilibrium between blood and saliva across salivary membranes, saliva analysis provide a comprehensive chemical characterization of exercise-related oxidative stress indicators and promises a new possible analytical approach to monitor physical exercise, in alternative to traditional blood and urine assays.

In this study we analyzed lactate, uric acid, carbonyls and isoprostanes in stimulated saliva samples in order to monitor oxidative stress in swimmers athletes performing a VO2 cycle ergometer test. For this purpose, ten healthy volunteers underwent incremental exercise on a cycle ergometer, at constant 60 rpm, with increment of about 25 W every minute until voluntary exhaustion or impossibility to maintain current workload. Stimulated saliva samples were collected 5 minutes before the exercise (t_0), at the maximum intensity (t_{max}) and 2.5, 5 and 10 minutes after the end of the test. Peripheral capillary oxygen saturation, electrocardiogram, heart rate, blood pressure, ventilatory equivalent to oxygen and carbon dioxide values were continuously monitored during all the entire experiment.

The results showed a clear increase of salivary metabolites levels during the exercise because of the increase of work load, whereas a sharp decrease, approaching baseline values, of these compounds was observed in the recovery phase.

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O2 BIO2

DROPLET MICROFLUIDICS FOR THE EFFECTIVE DETECTION OF MICRORNA

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MicroRNAs (miRNAs) are short, single-stranded, non-coding RNA molecules which act as crucial post-transcriptional regulators of gene expression, thus implicating many fundamental cellular processes such as cell proliferation, apoptosis, migration, differentiation, and invasion [1]. The detection of miRNAs, especially the abnormal miRNA expression, plays a critical role because of their contribution to serious human diseases [2]. Particularly, miRNAs in peripheral blood have recently been identified as significant and predictive biomarkers for liquid biopsy. Therefore, the development of effective miRNA detection methods is strictly required for clinical diagnosis. miRNAs show great challenges in detection due to their extremely short length (~22 nucleotides), very low concentration in biological fluids, and sequence homology [3]. The conventional approaches for nucleic acid detection, based on Polymerase Chain reaction (PCR) technique, suffer from low detection sensitivity, they are not inclined to directly detecting short RNAs and hardly allows discriminating between miRNA family members with very similar sequences. Recently, isothermal circular strand displacement polymerization (ICSDP) has emerged as a powerful method for the quantification of nucleic acids and for the utilization in developing miRNA assays. Compared to the standard protocols, isothermal amplification can be performed at a constant temperature cycling and it is able to reveal short-chains RNA and DNA with high sensitivity and specificity [4].
New digital bioassays represent an innovation in high throughput analysis since the target is partitioned into separate microreactors able to give ON/OFF signals, depending on whether the microreactors have zero or a single target entity. In a digital approach, the quantification of nucleic acids is performed without calibration standard curves or endogenous controls, and it enables the effective detection of analytes with low concentrations [5]. Typically, digital bioassay requires the development of microfluidic devices to obtain controlled droplet volumes, since the variation in the rector volume causes biases in the estimation of the target concentration. Droplet-based microfluidics offers many important advantages in biomolecular detection compared to continuous microfluidics, including reduced analysis time and sample volume, simplified automation of analytical procedures and integration of different functions in a single device.
The research work describes the development of a new microRNA detection method based on the combination of molecular beacon (MB) fluorescent probe with ICSDP amplification and droplet microfluidics. In particular, a specific configuration of the droplet microfluidic system compatible with a digital detection approach has been designed. The microfluidic

device generates spherical droplets with a nanoliter volume, which acts as a single chemical reactor individually analyzed. The bioassay allows discriminating between the target sequence (miR-127-5p), involved in osteoarthritis (OA) disease, and the blank signal of a mix solution of ICSDP reagents, without miRNA sequences, for 0 min and for 30 min. An increase of fluorescence signal has been observed after 30 min of the ICSDP amplification, by highlighting that the reaction takes place. Also, the discrimination of target miRNA sequence with a control unrelated sequence, operating at the nanomolar concentration, has been performed for 30 min. The experiments allowed to demonstrate that designed MB can selectively recognize the target sequence, which is amplified and detected.
These preliminary data provide promising perspectives for the picomolar or even femtomolar detection of microRNA under an experimental configuration compatible with the implementation of a microfluidic-based digital detection assay through MB-based ICSDP amplification.

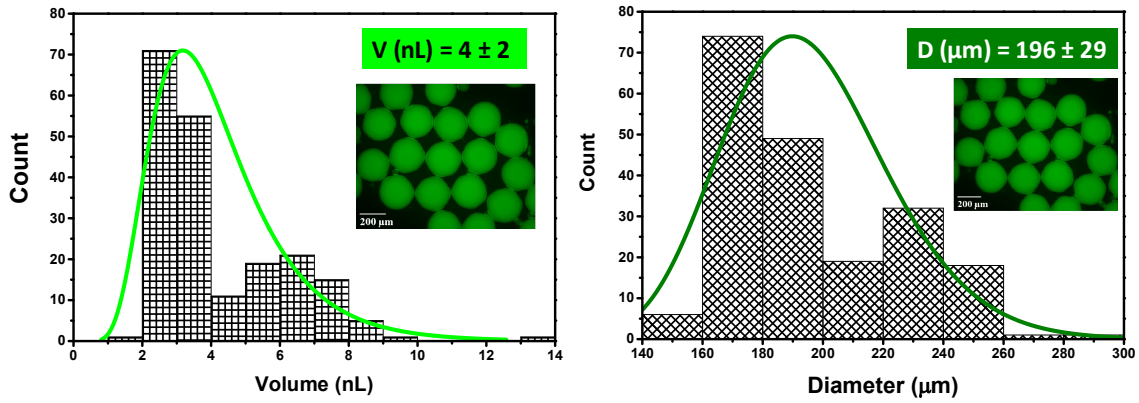

Figure 1. Size and volume characterization of single spherical droplets in the microfluidic device for the miRNA detection.

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O3 BIO2

FLUORESCENT SENSORY CORE-SHELL PARTICLES FOR SELECTIVE DETECTION OF SPHINGOSINE 1-PHOSPHATE AND PHOSPHATIDIC ACID

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Sphingosine 1-Phosphate (S1P) is a bioactive sphingolipid with broad range of activities coupled to its role in G-protein coupled receptor signaling [1]. Monitoring of both intra and extra cellular levels of this lipid is challenging due to its low abundance and lack of robust affinity assays or sensors. We here report on fluorescent sensory core-shell molecularly imprinted polymer (MIP) particles responsive to near physiologically relevant levels of S1P and the S1P receptor agonist Fingolimod Phosphate [2] (FP) in spiked serum samples. Imprinting was achieved using FP(TBA) or Phosphatidic Acid (DPPA(Na)) as templates in combination with a polymerizable nitrobenzoxadiazole (NBD)-urea monomer with the dual role of capturing the phospho-anion and signalling its presence. The monomers were grafted from ca 300 nm RAFT-modified silica core particles using ethyleneglycol dimethacrylate (EGDMA) as crosslinker resulting in 10-20 nm thick shells displaying selective fluorescence response to the targeted lipids S1P and DPPA in aqueous buffered media. Potential use of the sensory particles for monitoring S1P in serum was demonstrated on spiked serum samples, proving a linear range of 8-60 μM and a detection limit of 5.6 μM, a value slightly exceeding the plasma concentration of the biomarker.

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O1 EAC4

REVIEW OF AMBIENT PARTICULATE MATTER OXIDATIVE POTENTIAL MEASURED IN ITALY WITH ACELLULAR ASSAYS

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An emerging hypothesis in the field of air pollution is that oxidative stress is one of the important pathways leading to adverse health effects of airborne particulate matter (PM). Therefore, the oxidative potential (OP) - defined as the capacity of PM to oxidize target molecules generating reactive oxygen species (ROS) - has been proposed as a biologically relevant metric for assessing PM toxicity [1,2].
This work reviews the OP values measured to date in Italy, with the aim to provide a picture of the spatial and seasonal variability of OP in Italy, and give an insight into sources, processes and effects of meteorological conditions.
The paper summarizes the results obtained with the most common OP acellular assays based on target antioxidants simulating the PM−cell interaction generating ROS. The dithiothreitol (OP^{DTT}) and ascorbic acid (OP^{AA}) are based on low-cost spectrophotometric UV-Vis measurements of the depletion rate of DTT and AA, while the dichlorofluorescein assay (OP^{DCFH}) allows for the detection of PM–induced ROS via fluorescence spectroscopy [1].
The reviewed data concern different sites located in Continental and Peninsular areas, as shown in Figure 1.

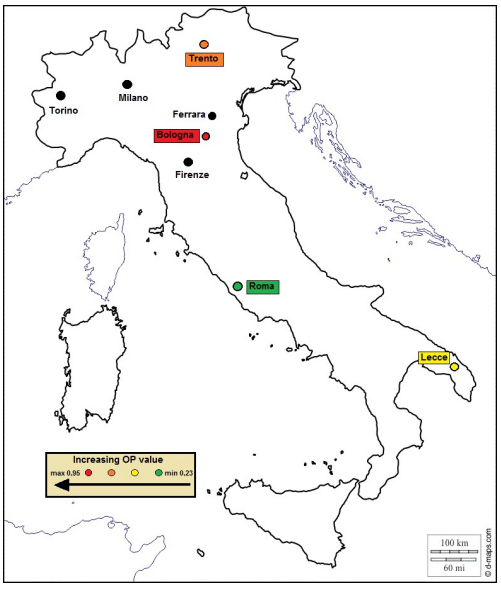

Figure 1. Location of the investigated sites reviewed in the paper. Coloured points describe the OP^{DTT} measured average responses (nmol min^{-1} m^{-3}).

O1 EAC4

Most of the available data were measured with the DTT assay. Overall, it provided mean OP^{DTT}_V values (volume normalized responses) ranging from 0.22 ± 0.18 to 0.95 ± 0.18 nmol min^{-1} m^{-3}. The AA assay has been used on PM samples collected at Bologna and Lecce and produced OP^{AA}_V responses varying from 0.24 ± 0.2 to 1.41 ± 0.2 nmol min^{-1} m^{-3}. The DCFH assay has been used in two studies to obtain OP values from 1870 ± 1861 µg ZYM/mg PM to 14882 ± 1861 µg ZYM/mg PM.

Overall, our synthesis indicates a generally greater PM OP in Po Valley, mainly related to emission sources and atmospheric conditions.

Moreover, on the basis of our observations, the three OP assays differ in the association with PM chemical composition, in seasonality and particle size distribution, even if they are sensitive to the same redox-active species in PM samples.

Another important outcome of our study is the identification of major species and sources that are associated with ROS activity. Water-soluble transition metals (e.g., Fe, Ni, Cu, Cr, Mn, Zn and V) and water-soluble organic carbon (WSOC) showed consistent correlations with the PM oxidative potential across different urban areas and size ranges.

The major PM sources associated with these chemical species include residual/fuel oil combustion, traffic emissions, and secondary organic aerosol formation, indicating that these sources are major drivers of PM-induced oxidative potential.

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O2 EAC4

SIMULTANEOUS MULTICLASS PRE-CONCENTRATION AND HPLC-MS/MS QUANTIFICATION OF ALGAL TOXINS IN ENVIRONMENTAL WATERS

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Global warming and anthropogenically-induced eutrophication of aquatic ecosystems are linked with increased frequency and magnitude of algal reproduction and thereby potentially harmful algal blooms. Marine biotoxins possess notable structural and physico-chemical properties different from each other. Domoic acid (DA) is a neurotoxic tricarboxylic amino acid, belonging to the class of kainoids, able to interfere with neurotransmission mechanisms by irreversible binding to glutamate receptors.
Okadaic acid (OA), a member of a group of molecules called polyketides, is an algal toxin responsible for diarrheic shellfish poisoning.
Microcystins (MCs) are hepatic cyanotoxins which contain five fixed amino acids and two variable amino acids (X and Y) in positions 2 and 4, which characterize each MC.
Increasing concern regarding the presence of algal toxins in environmental waters has resulted in the need for reliable analytical methods for their monitoring at nanograms per litre levels. [1]
Aim of the present study is to develop a simple and sensitive method for the simultaneous screening, detection and quantification of several classes of algal toxins at environmental concentration levels, using SPE followed by HPLC-HESI-MS/MS.
Pre-concentration tests were undertaken on tap water samples (250 mL) enriched with 0.8 µg L^{-1} of each analyte (DA, OA, MC-LW, MC-LR, MC-RR, MC-YR) using a carbonaceous sorbent material, recently proposed by our research group, HA-C@silica [2], evaluating different parameters, such as sorbent amount, extraction pH, composition and volume of the eluent. Adsorption is pH independent for all the analytes except for DA, which needs acid conditions. Therefore, the pre-concentration was carried out at pH close to 3, using 400 mg of HA-C@silica packed in 3 mL SPE tubes. MeOH with or without different percentages of Formic Acid (FA, 0-10% v/v) or Ammonia (NH_3, 0-5% v/v) was tested as eluent. The results highlighted that a single elution (2.5 mL) with MeOH 5% v/v FA provided recovery higher than 75% for all the analytes except for MC-RR (<20%). This toxin is strongly retained by the sorbent material probably due to the presence of an aromatic structure and polar groups, as highlighted by XPS analysis. So, further tests are now on going to assess the performance of different eluents and sorbent materials.
After SPE, algal toxins were separated and quantified by HPLC-HESI-MS/MS. Three reversed phase chromatographic columns were tested (Zorbax Eclipse Plus C18, 4.6 mm × 100 mm, 3.5 µm; Zorbax Eclipse Plus C18, 2.1 mm × 50 mm, 1.8 µm; Adamas C18-B 2.1 mm × 150 mm,

3.0 µm) and better chromatographic separations were obtained by the last one, using a gradient elution program with water and ACN both containing 0.5% v/v FA.
The quantitative analysis of the target compounds was performed in multiple-reaction monitoring (MRM) mode, using the two most intense and characteristic precursor/product ion transitions of each compound obtained from the MS/MS. The ionisation is performed in positive mode and the single protonated molecular ion $[M+H]^+$ is observed as precursor ion for all the analytes, except for MC-RR. This MC presented doubly protonated $[M+2H]^{2+}$ as precursor ion, because it contains two arginine residues in its molecular structure.
The main figures of merit - selectivity, sensitivity, linearity, recovery and precision – are evaluated and pre-concentration tests on lake and standing water are in progress to investigate the applicability to different environmental matrices.

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O3 EAC4

DEVELOPMENT OF AN ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH HIGH RESOLUTION MASS SPECTROMETRY METHOD FOR THE SCREENING OF CIANOTOXINS CONTENT IN DRINKING WATER SAMPLES

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More and more frequently waters intended for human consumption, for recreational, drinking, fishing or agricultural purposes, were affected by unusual cyanobacteria proliferation.
About 40 of the 150 known *genera* of cyanobacteria are able to produce, in particular conditions, secondary metabolites (called cyanotoxin) that are different for chemical structure and for toxic effects; unfortunately the studies about their toxicity are scarce and a linear correlation between the presence of a cyanobacteria bloom and the production of cyanotoxins does not exist, for the best of our knowledge.
In this context, it becomes important to develop analytical methods that allow the identification and, if possible, the quantification of as many cyanotoxins as possible.
This work presents the development of an analytical method to detect simultaneously 21 cyanotoxins of different classes (including 12 Microcystins, 5 Microginins, 2 Cyanopeptolins, and 2 Anabaenopeptins) using an ultra performance liquid chromatograph coupled with a high resolution mass spectrometer.
Microcistins are the most diffused cyanotoxin in Europe and World; they are monocyclic heptapeptides with an uncommon aminoacid ADDA, that are able to inhibit the protein phosphatase 1 (PP-1) and 2A (PP-2A) generating hepatotoxic effects [1].
Anabaenopeptide and Cyanopeptolins are cyclic non ribosomal oligopeptides produced by a broad range of cyanobacterial species that inhibit the serine proteases and the protein phosphatases, responsible for the regulation of several vital physiological and metabolic processes, but the studies about their ecological toxicity are scarce [2].
Microginins are linear peptides, characterized by N-terminal β-amino-α-hydroxy-decanoic or octanoic acid that inhibit zinc-containing metalloproteases; thirty-one different microginins had been isolated and fully characterized [3].
A chromatographic gradient was employed using acetonitrile and water as mobile phases, both containing 10 mM formic acid, and an Acquity UPLC system (Waters) equipped with an Acquity UPLC HSS T3 column (2,1 mm ID x 100 mm, 1,7 µm, Waters) at 40°C.
UPLC system was coupled with a XEVO G2S Q-TOF mass spectrometer (Waters) and the experiments have been carried out with a full scan 50-1200 in MS^E mode, positive ionization and resolution mode, with a scan time of 0.1 s.

O3 EAC4

This particular acquisition mode allows to alternate low collision energy (MH^+ or MH^{n+} signal) and elevated collision energy (fragment signal) during the same experiment.
In this work, a collision energy ramp was optimized to obtain an optimal fragmentation for all the analytes.
Appling the previously described experimental conditions to a drinking water sample, we are able to identify 4 of 21 selected cyanotoxins and simultaneously, analyzing all the detected signals, to reveal the presence of non target compounds.
The method resulted robust, in terms of repeatability, reproducibility, linearity and detection limits that are at least 20-fold lower than the guideline value proposed by WHO for drinking water.
Moreover, this method allows the simultaneous identification of target and non-target compounds, in short time of analysis (16 minutes) and low injection volume (10 µl) allowing to detect the presence of other compounds potentially harmful to human health.
The analytical method proposed in this study can be applied to assure the prevention and management of emergencies caused by the proliferation of cyanobacteria and the cyanotoxins production in water for human consumption.

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O1 SB4

SENSING OF FURFURAL BY MOLECULARLY IMPRINTED POLYMERS WITH ELECTROCHEMICAL TRANSDUCTION

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The determination of furanic compounds at different concentration levels in an aqueous medium, as for example beverages, is becoming a crucial task in food control, because of the relevance of these substances for food flavor and also for their possible toxic and carcinogenic effects on the human beings. For these reasons, their determination by fast, easy and low-cost methods is of interest. In the present investigation, the possibility of using molecularly imprinted polymer (MIP)-modified electrochemical sensors for the detection of a particular furanic derivative, furfural (2-furaldheide (2-FAL)), in aqueous media is examined. Chemical sensors are based on the strict integration of a receptor with an instrument able to generate a signal upon the combination of the receptor with the substrate. Both the characteristics of the binding reaction, in particular the affinity constant, and that of the transductor, for example the sensitivity, are of overwhelming relevance for determining the performance of a sensor.

In the present work, we examine a solid synthetical receptor (MIP) obtained by non-covalent molecular imprinting of 2-FAL connected with an electrochemical transduction method. MIPs have been widely demonstrated to be advantageous with respect to the biological receptors, in particular in the field of sensing, in terms of reproducibility, fast and low cost development, stability in time and possibility of application in non physiological conditions [1, 2]. A drawback consists in the heterogeneity of the binding sites, particularly when the MIPs are synthesized by non covalent bulk procedure [2], which on the other hand could allow to perform measurements at different concentration levels. The detectable concentration level, in turn, depends on the sensitivity of the detection technique employed. Various MIP electrochemical sensors have been developed in the last years [3,4] for the determination of a number of small molecules. The voltammetric sensor here proposed is particularly convenient because of the low cost, good reproducibility and easy preparation [4]. It is composed of a screen printed cell (SPC) with graphite ink working and auxiliary electrode, and silver ink quasi-reference electrode, obtained by the screen printing technique, and of a MIP layer deposed over the whole cell. The signal is generated by the substrate itself, i.e. 2-FAL, since it is electroactive [5]. The signal is the peak current (i_p) obtained by square wave voltammetry (SWV), which is directly proportional to the analyte concentration in the polymeric layer near the working electrode. This in turn depends on the concentration in the solution phase, the binding to the MIPs taking place according to the Langmuir adsorption isotherm [2]. A typical voltammogram is reported in Figure 1b. The sensitivity of the SWV electrochemical detection depends on the experimental condition, for example the acidity of the solution, and on the SWV conditions. These have been optimized

by the Experimental design (ED) method, in order to have a maximum peak current, and at the same time a minimum background current.
The sensitivity was improved about 10 times by changing the SWV conditions from Estep=0,01 V, Epulse=0,025 V, f=25, to Estep=0,03 V, Epulse=0,065 V, f=28 s^{-1}, allowing to lower the detection limit of a factor of ten.

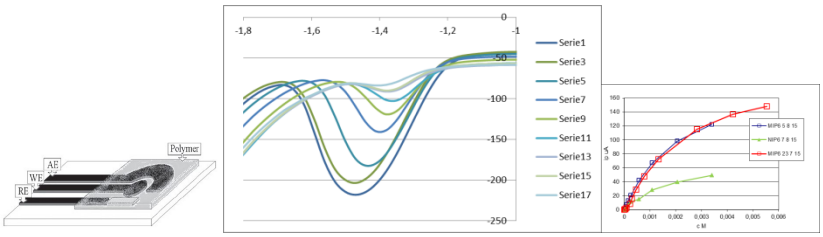

Figure 1. a) Schematic view of SPC modified with MIP. b) SWV voltammograms of 2-FAL in NaCl 0.1 M at MIP-modified sensor at Estep=0,03 V, Epulse=0,065 V, f=28 s^{-1}.Concentration of 2-FAL from 2 10^{-4} to 5 10^{-3} M. c) Binding isotherms of MIP and NIP modified sensor in water at pH=6 NaCl 0.1 M.

At optimized conditions the electrochemical sensor shows an LOD of about 6*10^{-6} M for 2-FAL at neutral pH in an aqueous 0.1 M NaCl solution and in a white wine real sample.

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O2 SB4

EXPANDING THE CAPABILITIES OF WASH-FREE, ELECTROCHEMICAL DNA SWITCHES FOR THE DETECTION OF DIAGNOSTIC ANTIBODIES IN AUTHENTIC HUMAN SAMPLES

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A fast and precise diagnosis of infectious diseases -and thus the initiation of their treatment- is paramount to achieve a positive course of the disease. Here we present a novel wash-free, electrochemical sensor that measures the levels of specific antibodies directly at the point-of-care and during the few minute timeframe of a typical doctor´s appointment.
Our platform, named E-DNA sensors, consists of a short, double-stranded nucleic acid attached by one end via a flexible linker to a gold electrode and modified on the other with a redox reporter (methylene blue) and an epitope/antigen (Fig.1-A). The binding of the specific antibody reduces the rate of electron transfer from the methylene blue to the electrode, generating a measurable change in the electrochemical signal, which we use to quantify the target concentration. E-DNA sensors are reagentless, single-step, and selective enough to deploy in whole blood serum, making them excellent candidates for point-of-care applications. In addition, the equilibration time constant of antibodies, generally in the order of few minutes, matches our “timeframe-goal”.
We demonstrated that we can include as recognition elements of our sensing platform not only linear epitopes but also full-size antigens (<70 kDa, Fig.1-B). Taking advantage of this modularity, we designed five sensors employing different epitopes from the HIV-antigen gp41 and one using the full p24 antigen. After confirming the immunogenicity of each epitope/antigen, we simultaneously detected multiple HIV-specific antibodies directly in human samples. We achieved the same clinical sensitivity and specificity as those of ELISAs and lateral flow immunoassays, being able not only to discriminate between healthy and HIV-positive patients, but also to differentiate early-infected from late-infected individuals (Fig.1-D).
The E-DNA platform appears a versatile, clinically sensitive and specific method for the rapid, single-step detection of antibodies at the point-of-care.

O2 SB4

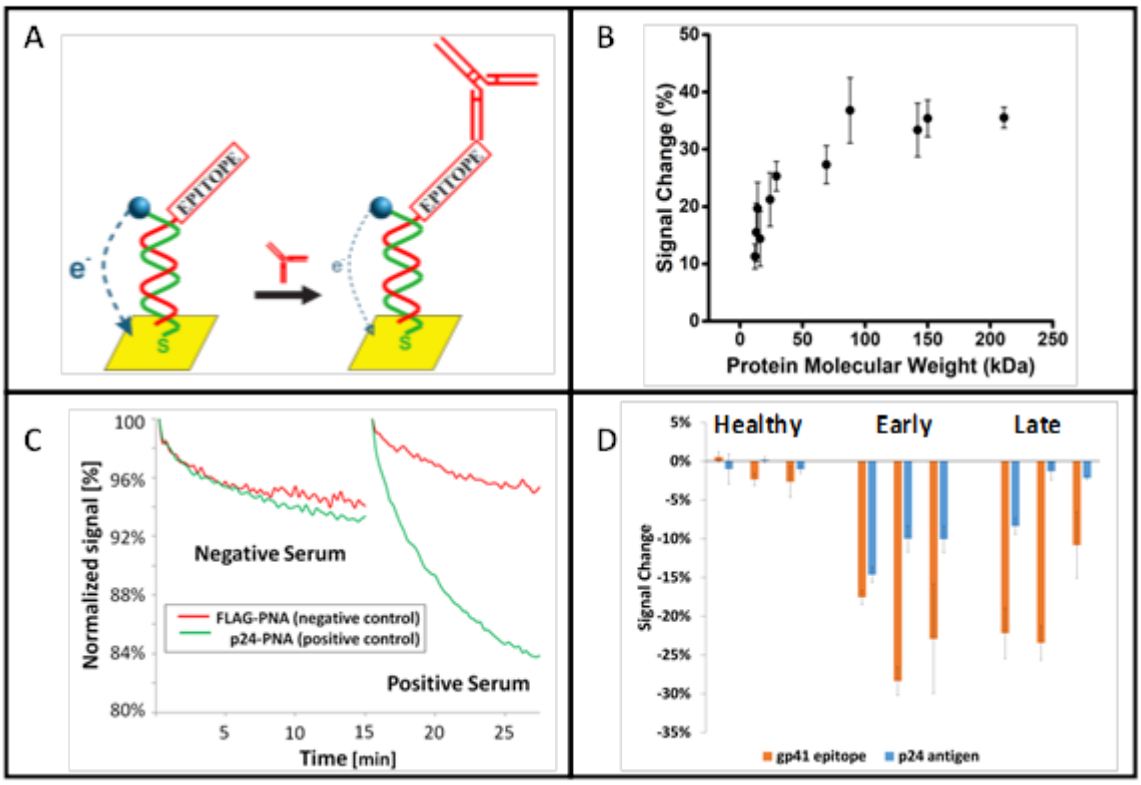

Figure 1. A) Cartoon of the E-DNA electrochemical antibody sensor. B) Impact of the antigen size on the binding-induced signal change. C) Continuous measurement of HIV-positive and HIV-negative serums using E-DNA sensors. D) Discrimination of healthy, early-infected and late-infected HIV-positive patients using E-DNA sensors.

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O3 SB4

AMPEROMETRIC BIOSENSOR BASED ON LACCASE PHYSICALLY ENTRAPPED ON A POLYTHIOPHENE–MODIFIED SCREEN PRINTED ELECTRODE FOR RAPID DETECTION OF TOTAL POLYPHENOL CONTENT IN FOOD MATRIX

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The communication between enzymes and electrode surfaces is of fundamental importance in biosensor development.
Conductive polymers, such as polyaniline, polypyrrole and polythiophene have been very often employed as electrode modifier to allow an efficient enzyme wiring. Their (semi)conductive properties, biocompatibility and versatility, have made these materials very interesting in this field since their discovery in the late 1980s. Furthermore, the electrochemical polymerization allows a direct deposition on the electrode surface and it is much faster than a time and reagents-consuming chemical synthesis.
Despite these features, polythiophene application in biosensing has only partly been seen until now. It has been used mainly in molecularly imprinted polymers, in supercapacitors and for enzyme immobilization. One of the problems that can affect the enzyme interacting with this kind of polymer is the need to use organic solvents to solubilize the monomers and to reach the high potentials requested for the monomer oxidation in electrosynthesis [1]. Further, during the polymerization, the upcoming polythiophene structure can be damaged by the high potential applied (polythiophene paradox).

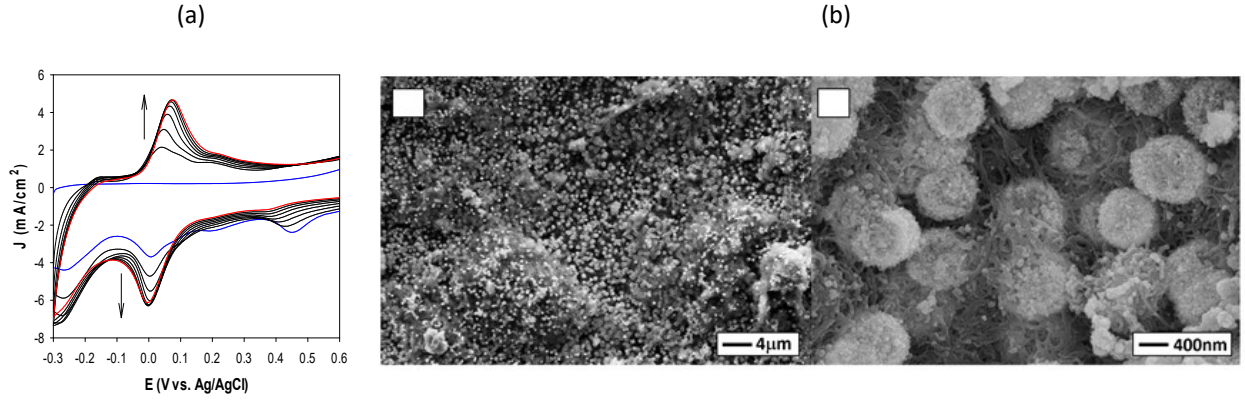

Figure 1. (a) Cyclic voltammetry for electropolymerization of 3-metoxy-thiophene in aqueous solution, (b) SEM images of electropolymerized electrodic surface at different magnifications.

The aim of this work was the evaluation of an innovative immobilization method based on electrosynthetized polythiophene for the development of biosensor. The method has been tested with laccase in order to evaluate the possibility to use polythiophene as

immobilization matrix, preventing enzymatic denaturation phenomena which would affect the biosensor performance in terms of sensitivity, linear range, and stability. To this end, the biosensor proposed herein has been prepared in mild conditions by using a graphite SPE electrode, modified by electropolymerization in an aqueous solution of 3-methoxythiophene (Figure 1(a)) in the presence of laccase performing cyclic voltammetry in drop mode [2]. The polymer film was characterized by scanning electron microscopy (Figure 1(b)) and impedance spectroscopy. The calibration plot of the biosensor showed a linear response in the concentration range from 7 to 110 µM expressed as catechol, with a limit of detection of 2.5 µM. The method exhibited good selectivity, stability and reproducibility for detecting polyphenols in foodstuffs.

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O4 SB4

AN ENZYME-LINKED OLIGONUCLEOTIDE ARRAY FOR THE ELECTROCHEMICAL DETECTION OF AFLATOXIN B_1

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Mycotoxins are a problematic and toxic group of small organic molecules that are produced as secondary metabolites by several fungal species that colonise crops. With wide ranging structural diversity of mycotoxins, severe toxic effect caused by these molecules and their high chemical stability, the requirement for robust and effective detection methods is clear [1]. The most relevant group of mycotoxins is that of aflatoxins, carcinogenic products belonging to the *Flavus*, *Parasiticus* and *Nomius* species of the genus *Aspergillus* [2]; among these, aflatoxin B_1 (AFB_1) is a potent human carcinogen (first hazard class in accordance with the classification of the International Agency for Research on Cancer) [3].
In this work, an electrochemical enzyme-linked oligonucleotide array to achieve simple and rapid multidetection of aflatoxin B_1 (AFB_1) is presented. The assay is based on a competitive format and disposable screen-printed cells (SPCs). Firstly, the electrodeposition of poly(aniline-anthranilic acid) copolymer (PANI-PAA) on graphite screen-printed working electrodes was performed by means of cyclic voltammetry (CV). Aflatoxin B_1 conjugated with bovine serum albumin (AFB_1-BSA) was then immobilized by covalent binding on PANI-PAA copolymer. After performing the affinity reaction between AFB_1 and the biotinylated DNA-aptamer (apt-BIO), the solution was dropped on the modified SPCs and the competition was carried out. The biotinylated complexes formed onto the sensor surface were coupled with a streptavidin-alkaline phosphatase conjugate. 1-naphthyl-phosphate was used as enzymatic substrate; the electroactive product was detected by differential pulse voltammetry (DPV). The response of the enzyme-linked oligonucleotide assay was signal-off, according to the competitive format. A dose-response curve was obtained between 0.1 ng/mL and 10 ng/mL with a limit of detection of 0.086 ng/mL. Finally, preliminary experiments in maize flour samples spiked with AFB_1 were also performed. From the obtained results, the developed analytical tool has proven itself to be applicable for screening field analysis.

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O5 SB4

MINIATURIZED ALL-INCLUSIVE BIOSENSOR BASED ON CHEMILUMINESCENT LATERAL FLOW IMMUNOASSAY FOR FECAL HEMOGLOBIN DETECTION: A HOME-MADE TEST FOR COLORECTAL CANCER SCREENING

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Colorectal cancer is the second leading cause of malignant death and the participation rate to screening programs based on invasive endoscopic diagnostic tests is very low. As an alternative, non-invasive stool testing based on the detection of fecal occult blood represents a valid approach for a rapid screening. However, the widespread guaiac-based test (GFOBT) is affected by a variety of interferences, thus frequently yielding false-negative and false-positive results and requiring the patient to follow a specific pre-test diet. The use of an immunoassay for detecting hemoglobin in stools could overcome these limitations. 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 ready-to-use strips of cellulose-based materials containing dry reagents that are activated upon fluid sample application. Using enzymes as tracers, coupled with chemiluminescence (CL) detection, it is possible to obtain quantitative information and reach high detectability.
Herein, we report the development of a simple, rapid and accurate biosensor based on a CL-LFIA method applied for quantitative detection of hemoglobin in stool samples, using the smartphone BSI-CMOS photocamera as a light detector [1,2]. The biosensor is based on a competitive immunoassay using peroxidase (HRP)-labeled anti-hemoglobin antibody, which is detected, upon adding the luminol/enhancer/hydrogen peroxide-based CL substrate, by means of a smartphone camera for digital imaging and a specific application for data handling. Using a 3D printer, simple accessories were developed to turn the smartphone into a biosensing device. Since CL system employs labile enzyme that makes it hard to routinely use them for on-site applications, it is proposed to entrap the HRP-labeled anti-hemoglobin antibody into a pullulan-based tablet, which allows to enhance the long-term stability of the enzyme and also to simplify the assay procedure. Indeed, these tablets dissolve rapidly upon addition of the samples, making the test very easy to be performed on site. Moreover, new geometries and smaller dimensions of the LFIA membrane are evaluated, in order to find a good compromise between the execution time, the compactness of the device and the analytical performances of the immunoassay. The developed method is simple and fast (15-min total assay time) (Figure 1) and it allows to detect even small traces of hemoglobin in fecal samples, down to 4 pmol (Figure 2). When compared with the conventional GFOBT the

assay is able to detect lower concentration of blood allowing an early diagnosis. This biosensor could be very useful for frequent self-screening providing a very effective tool for colorectal cancer prevention.

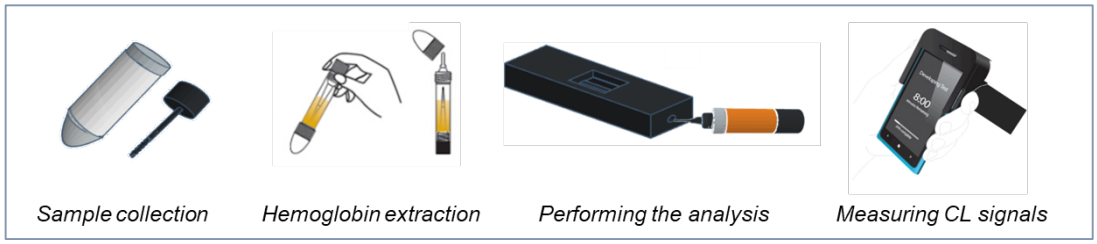

Figure 1. Schematic procedure for the CL-LFIA based method for hemoglobin quantification in feces sample

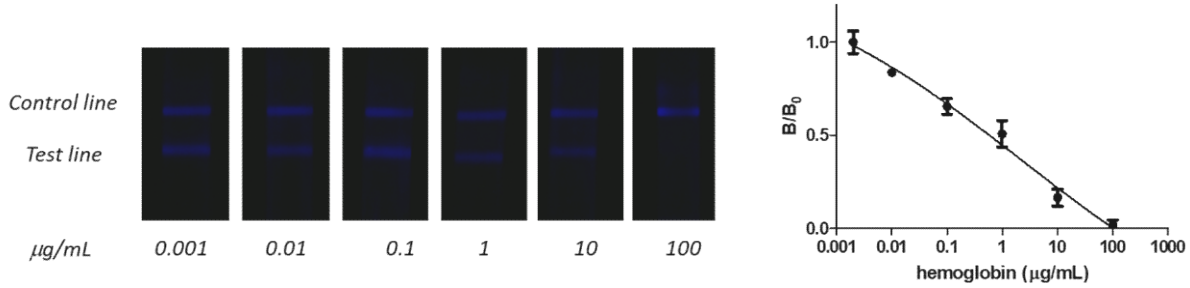

Figure 2. Chemiluminescent images obtained for different haemoglobin concentration and the relative calibration curve

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O6 SB4

STAPHYLOCOCCAL PROTEIN A AS POWERFUL AND VERSATILE KEY PLAYER FOR PAPER-BASED BIOSENSORS DEVELOPMENT

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In the last decades, the huge need to perform analytical tests outside of the laboratory caused the exponential diffusion of the so-called "point-of-use" tests. These tests must be portable, affordable, user-friendly and allow to perform the analysis directly *in situ*.
Among these systems, the paper-based biosensor, also known as Lateral Flow Assay (LFA), has become one of the most successful analytical format for point-of-use testing.
The lateral flow assay technology is also very versatile as combines a number of variants such as test formats, recognition elements, signal reporters, and detection systems. All these features make LFA particularly attracting for different fields, including clinical, veterinary, food safety, forensic and environmental analysis [1].
Most existing LFAs exploit the unique properties of the antigen-antibody interaction to enable high sensitive and selective analysis. The most popular detection is the visual one based on colored probes, and colloidal gold nanoparticles (GNPs) are the most widely employed probes in color-based lateral flow assays [1-3].
A critical point for the successful development of a visual paper-based biosensor is to obtain a stable and efficient labelled conjugate between antibodies (Ab) and GNPs.
Since proteins - and particularly, immunoglobulins (IgG) - spontaneously adhere to the surface of GNPs capped by citrate through several types of non-covalent interactions [4], the direct adsorption of antibodies onto citrate-capped GNPs is the most commonly used method to prepare GNP-Ab probes. As a consequence, the analytical antibodies are randomly adsorbed onto GNPs and it has been estimated that only ca. 25 % of the passively adsorbed antibodies are able to bind the antigen [5].
A more efficient GNP-Ab conjugate can be obtained through the use of a binding mediator between GNPs and analytical antibodies themselves.
Staphylococcal protein A (SpA) is a high stable surface receptor with a molecular weight of 42 kDa. It is known that SpA is able to bind IgG from several mammalian species, with high affinity for the Fragment crystallizable portion of IgG. Therefore, the use of SpA as biochemical mediator allows to suitably orientate the specific antibody, yielding to a highly active probe (more than 90% of the antibodies bound through protein A are available for the binding [5]).
Moreover, it is possible to use the SpA as recognition element in point of care diagnostics for infectious diseases, where the goal is to detect the Immunoglobulins [6].
We exploited the affinity-based binding properties of the SpA for the development of robust and versatile paper-based biosensors. In this communication, the results of the study will be discussed pointing out the major advantages and drawbacks.

O6 SB4

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O1 BIO3

INNOVATIVE USES OF POLYDOPAMINE (PDA) IN THE FIELD OF (BIO)ANALYTICAL CHEMISTRY

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The history of dopamine (DA) investigation starts at the beginning of the 20th century with the chemical synthesis [1]. Later on, DA was identified in human body, together with the discovery of multiple functions as monomer or polymer [2,3]. The description of the DA metabolism in the brain and peripheral areas allowed the explanation of age-related synthetic decline [4], the recognition of pathologies associated with concentration anomalies of DA and subsequent associated-drugs application in several medical conditions [5]. Finally, there was the reawakening of synthetic dopamine at the beginning of the 21st century [6-8], when the non-enzymatic oxidation to *ortho*-quinone and the subsequent self-polymerization has generated several applications in medicine, (bio)analytical chemistry, and materials science [9-14]. The huge significance of endogenous Dopamine (DA) and the severe clinical conditions associated with DA concentration anomalies, including Schizophrenia, and Parkinson's disease, has generated more than 200 thousand scientific papers over the past 80 years. A more recent and intriguing outcome from this plethora of information is represented by the electrochemical and chemical reaction pathway for polydopamine (PDA) formation in aqueous solutions that requires the synthesis of 5,6-indolequinone (IQ) monomer from dopamine molecule [4,15]. Notably, the polymer growth is inhibited by low pH (below 4) and high-concentration of various electrolytes that impair the preliminary intramolecular cyclization of oxidized dopamine by decreasing the nitrogen nucleophilicity [6]. Since the pioneering investigations and application on dopamine polymerization by electrodeposition [6,7], and then by O_2/pH-induced oxidation [8], thousands of papers involving the PDA synthesis, study and application have been published. Notably, the last four years represent almost 80% of all the scientific production, underlining the enhanced interest arising from the versatile chemistry of this endogenous catecholamine and its complex polymerization mechanism. The redox potential of catechol moiety has been exploited to produce optically and catalytically active metal nanoparticles in situ. This feature responsible for the cross-linking of dopamine can be enhanced by chemical oxidants, UV, or microwave irradiation, influencing the coating of PDA at nanometric scale employed for a variety of physical, chemical and biological studies. However, this field of research is still young and challenging in application of PDA-coated surface to medicine, energy and industrial manufacturing, for example. In particular, a promising field of PDA research is the surface coating for molecular sensing and affinity separation for pharmaceutical studies and clinical applications, following the peculiar physicochemical properties of PDA, and the molecular immobilization and imprinting capability of this biopolymer. Here we report a survey of this demanding area of bioanalytical research, focusing on the state-of-art of PDA

applications for coating and imprinting, and offering a long-term vision for the capability of this polymer to be exploited to its full potential.

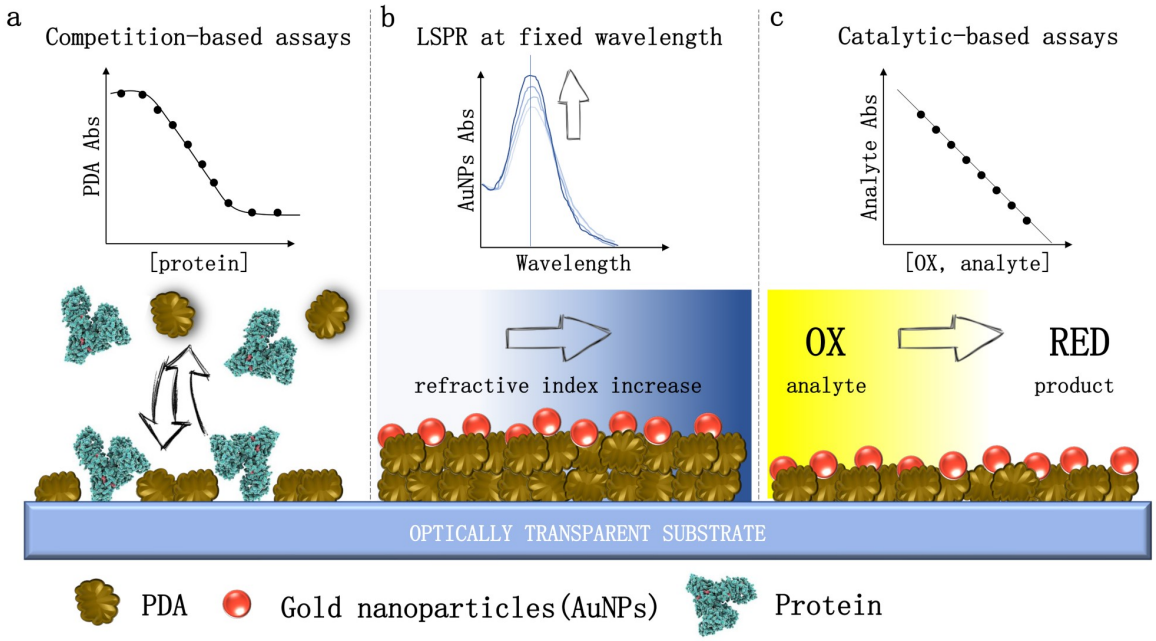

Figure 1. Innovative uses of PDA in the field of (bio)analytical chemistry. (a) Competition-based assay for quantification of total protein in biological fluids. (b) (LSPR)-based quantitative assay at fixed wavelength for applications in clinical, food, and environmental controls. (c) Catalytic-based assay for redox reactions. From reference [13].

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O2 BIO3

HIGH-FREQUENCY, REAL-TIME MEASUREMENTS OF PLASMA PHENYLALANINE IN SITU IN THE BODIES OF LIVE RATS USING AN ELECTROCHEMICAL APTAMER-BASED SENSOR

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The ability to measure small molecules in the body in real time and with seconds resolution would advance our understanding of physiology and medicine. Seconds-resolved measurements of metabolites in blood, for example, would describe metabolic fluxes and control with far higher precision and accuracy than current approaches, which require removal of samples from the body for later analysis. This ability could be crucial to reveal physiological differences in enzyme's level of activity in presence of a specific pathology, as a tumor, or in different regions of the body [1]. The real-time monitoring of metabolites could, in parallel, optimize treatment for patients affected by metabolic or neurological disorders [2]. Thus motivated, we have developed electrochemical aptamer-based (E-AB) sensors, a real time, seconds-resolved measurement platform that, as critical for operation in vivo, is reagentless, reversible, and selective enough to work in bodily fluids [3]. Here, we describe the development and characterization of an E-AB sensor against the aromatic amino acid phenylalanine and its adaptation to in-vivo measurement of this important metabolite. As the first step in this process we adapted a new selected phenylalanine-binding aptamer to the platform by attaching it to an electrode and modifying it with a redox reporter (methylene blue). We then characterized the sensor in vitro in undiluted whole blood, demonstrating its ability to respond rapidly and specifically to phenylalanine over the amino acid's metabolically relevant range. To ensure that the E-AB sensor works in vivo in the veins of live animals we used a drift correction scheme termed "Kinetic Differential Measurements" (KDM) based on the different square wave frequency dependence of E-AB signaling. Exploiting KDM approach, the resultant 200 µm-diameter sensor achieves 14-s-resolved measurements of plasma phenylalanine levels in vivo over the course of hours when emplaced in the jugular veins of live rats, thus providing an unprecedentedly high-precision view into the metabolism of phenylalanine and in the activity of phenylalanine hydroxylase (PAH).

O2 BIO3

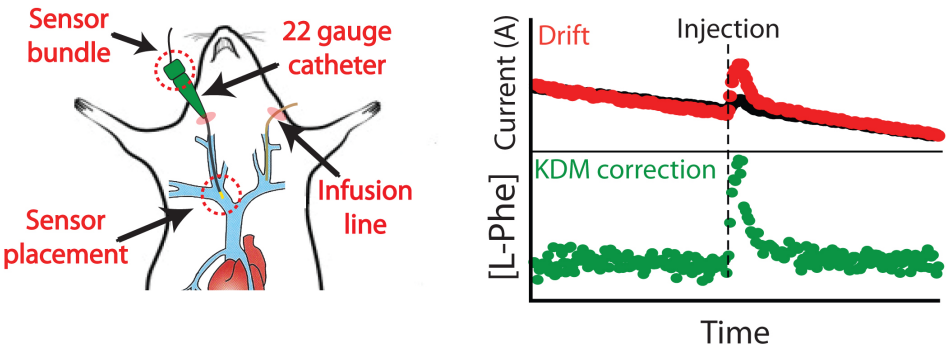

Figure 1. Indwelling E-AB sensors supporting the high-frequency measurements of plasma phenylalanine levels in situ in the living body.

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O3 BIO3

WHOLE CELL BIOSENSORS *vs* CELL-FREE BIOSENSORS FOR BIOANALYTICAL APPLICATIONS: A SIDE BY SIDE COMPARISON

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Whole-cell biosensors have been widely used for several applications as they provide useful information about the bioavailability, general toxicity, and bioactivity of a target analyte or a sample. During the last decade, thanks to the implementation of smartphones and other user-friendly light detectors, they have been also integrated into compact low-cost analytical devices. However, the scarce robustness of living cells still represents an issue and several immobilization methods have been developed for improving cell's shelf-life and obtain ready-to-use biosensors. More recently, cell-free transcription-translation (TX-TL) systems have been also proposed as a valid alternative. Conversely to whole-cell biosensors, TX-TL systems do not rely on living cells but rather include the biological machinery and energy source to express a reporter protein as consequence of target activation. Whole-cell and cell-free biosensors have become preferential alternatives to conventional analytical methods for rapid detection of analytes of environmental interest as they are cost effective and easy to implement into portable devices. The choice of reporter genes in biosensors, is also a key factor especially for on-site monitoring. A reporter gene is a gene which can be easily and quantitatively distinguished over a background of endogenous proteins. Several reporter genes have been widely employed to monitor cellular events associated to signal transduction, including the application in biosensors.
Here we compared three optical reporter categories, e.g., fluorescent, colorimetric and bioluminescent reporters in both whole cell biosensors and cell-free transcriptional and translational system for heavy metal and bacterial contamination in water. Particularly, green fluorescent reporters (GFP and deGFP), red fluorescent reporters (mCherry and mScarlet-I), colorimetric reporter (LacZ) and bioluminescent reporters (NanoLuc luciferase and lux operons from Aliivibrio fischeri and Photorhabdus luminescens) have been analysed. A comprehensive profile of the analytical performance, in terms of limit of detection (LOD), sensitivity, input/output dynamic ranges and response time, obtained with diverse optical reporters is reported (Fig.1).

O3 BIO3

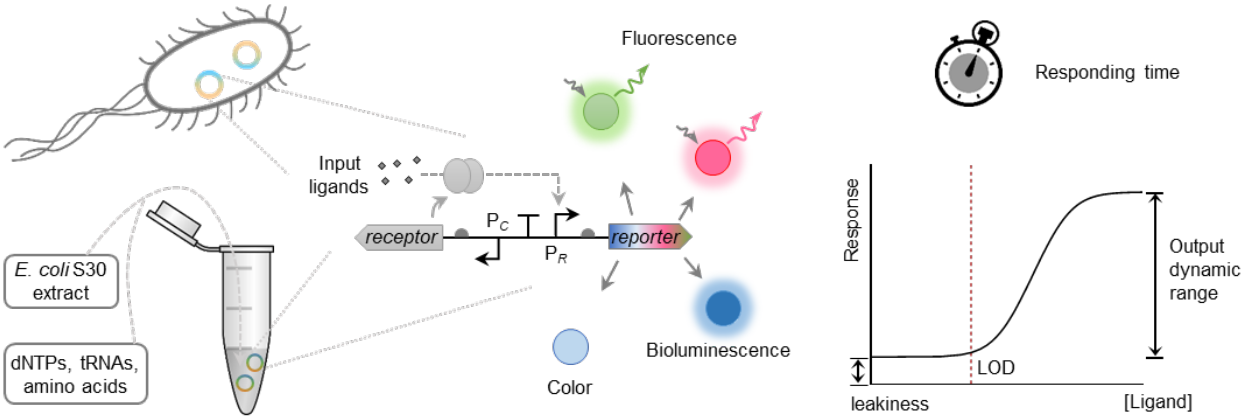

Figure 1. Schematic representation of the comparison of different reporter genes in whole cell and cell-free system

According to our results, enzymatic reporters are the best candidates. Especially NanoLuc luciferase showed the lowest LOD (50.0 fM of $HgCl_2$) within the shortest response time (30 min), proving its eligibility as reporter gene for rapid and sensitive on field monitoring.
Despite this, the selection of reporters needs to be a balanced compromise with other important factors, such as the background signals (higher in fluorescent gene), cost of the assays and need for substrate addition, especially for point of care and point of need applications.

O4 BIO3

TURN-ON CHEMILUMINESCENCE BIOASSAY FOR RAPID AND SENSITIVE QUANTIFICATION OF INTRECELLULAR H_2O_2 AND FOR ANTIOXIDANT SCREENING IN HUMAN LIVING CELL

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A new rapid (less than 1 hour) and simple effect-based bioassay for the selective measurement of intracellular H_2O_2 in live cells is reported. The bioassay relies on an adamantylidene - 1,2 - dioxetane probe containing an arylboronate moiety that in the presence of H_2O_2 is converted to the correspondent phenol. This triggers the chemiluminescent decomposition of the probe, which emits green light with high efficiency[1]. Under optimized conditions LOD and LOQ of 0.15 µM and 0.50 µM H_2O_2, respectively, have been obtained (corresponding to 3×10^{-11} and 1×10^{-10} moles of H_2O_2). Taking advantage of its high selectivity and low detection limit, the probe has been successfully employed for the quantification of intracellular H_2O_2 in living human endothelial, colon and keratinocyte cells exposed to different pro-oxidant stimuli (i.e., menadione, PMA and LPS). Imaging of living cells[2] clearly reveals the chemiluminescence emission from cells after pro-oxidant stimuli. Treatment of cells with antioxidant molecules leads to a dose-dependent decrease of intracellular H_2O_2 during biological processes. As a proof of concept, the bioassay has been used to measure the antioxidant activity of extracts from a *Brassica juncea* (oriental mustard) "Broad-leaf" selection, containing glucosinolates, isothiocyanates and other antioxidant molecules.

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(A) (B)

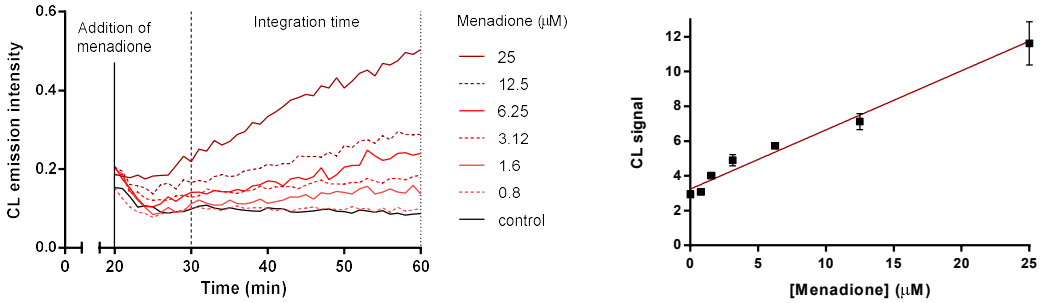

Figure 1. (A) Chemiluminescence kinetic profiles obtained for Caco-2 cells in the presence of the H_2O_2 CL probe and different concentrations of menadione. (B) Dose-response showing the correlation between the CL signal and the concentration of menadione. Each point represents the mean ± SD of three independent measurements.

The project was funded by Cariplo Foundation within the "Agroalimentare e Ricerca" (AGER) program. Project AGER2-Rif.2016-0169, "Valorizzazione dei prodotti italiani derivanti dall'oliva attraverso Tecniche Analitiche Innovative"-"VIOLIN".

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O5 BIO3

MOLECULARLY IMPRINTED NANOGELS COMBINED TO PLASTIC OPTICAL FIBRE FOR THE ULTRALOW DETECTION OF PROTEINS

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Molecular imprinted polymers (MIPs) are synthetic materials with entailed recognition properties, prepared by a template assisted synthesis [1], that can target a wide spectrum of analytes, ranging from small molecules to proteins [2] and can be shaped in formats from micron- to nano-dimensions. Exhibiting recognition properties similar to antibodies and natural receptors, but the robustness and integrability to sensing devices typical of the polymeric materials, MIPs are envisaged as a promising receptor element for sensing purposes. Moreover downsizing the MIPs to nanodimensions (nanoMIPs) [3] offers significant advantages in terms of binding kinetics, accessibility of the binding sites, homogeneity of the imprints, quasi-protein-sized dimensions, strengthening further their resemblance to natural receptors.
Here we explored the potential of MIP nanogels, characterized by solvent-responsive properties, in plasmonic sensing on a D-shaped plastic optical fibre (POF) [4], this latter chosen for the versatility of configurations offered, the easy and low cost of manipulation, the great numerical aperture, the large diameter, the possibility to withstand smaller bend radii than glass, the use of white light sources and the remote interrogation.
The POF platform was covalently derivatized with the nanoMIPs resulting in a nanoMIP-POF sensing platform with homogeneous surface coverage and responsivity for solvents. The nanoMIP-POF platform selectivity and sensitivity was investigated, showing the ability to detect the model protein at ultralow concentrations and with femtomolar LOD.

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O6 BIO3

QUALITY CONTROL OF RESIDENT STEM CELL BY HIGH PRESSURE LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

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In 2017 the Cell Factory, the first pharmaceutical industrial unit in Piedmont, received the AIFA (Agenzia Italiana del Farmaco) authorization to prepare adult stem cells for cell and gene therapies purposes.
Cell and tissue production must be done following the GMP (Good Manufacturing Practices) and GLP (Good Laboratory Practices) rules, exactly as for drug development. This is because the cell culture products have been recognized from European Union as drugs.
In particular, the cell factory produces resident stem cell for cell therapy in adult patients affected by acute liver failure (ALF).
The cells were cultured in presence of fetal bovine serum (FBS) and some cytokines, and a quality control is required to exclude the presence of anti-inflammatory drugs, such as ketoprofen, and pro-inflammatory agents, such as cytokines, a family of small proteins involved in immune system response.
The aim of the research was to develop analytical methods based on high pressure liquid chromatography (HPLC) and mass spectrometry (MS) to identify and quantify impurities traces in stem cells produced by the Cell Factory.
It was well documented that MS is one of the best tool to investigate and quantify presence (amount) of molecules in biological samples [1]. In particular, tandem mass spectrometry (tandem MS) is recommended to quantify small molecules [2]; instead, high resolution (HR) MS, such as the Orbitrap technology, is endorsed for intact proteins and peptide quali and quantification [3].
We started the quality control process with the quantification of ketoprofen in stem cells with ultra (U)HPLC (Nexera, Shimadzu) coupled through an ESI source to a tandem MS triple quadrupole (QTRAP5500, Sciex). Samples were spiked with ketoprofen d3 (internal standard) and combined with acetonitrile to allow protein precipitation. Sample were vortex mixed and centrifuged. Clear supernatants were placed into a vial and analyzed by UHPLC tandem MS.
To quantify ketoprofen in samples, three calibration curves were prepared. A solution of water/acetonitrile 1/1, FBS (fetal bovine serum) and growth medium were used respectively as matrix/solvent and compared in the study of matrix effect. The matrices were spiked with known concentration of ketoprofen and ketoprofen d3 (fixed). The column was a Kinetex sub 2 µm particle size (Phenomenex) and the elution was obtained used 0.1% formic acid in water and acetonitrile with a flow of 400 µL min^{-1}. The quantification of ketoprofen with tandem MS was done in MRM (multi reactions monitoring) following the transitions

O6 BIO3

253>209 *m/z* and 256>212 *m/z* for ketoprofen and ketoprofen d3 respectively. The obtained LLOQs (lower limits of quantitation) were 0.1 µg/L for calibration curve in solvent, 7.5 µg/L both for calibration curve in FBS and in growth medium. The analyzed samples did not show the presence of ketoprofen over LLOQ. No signal for the analyte molecule was detectable.
To control the presence of pro-inflammatory agents, cytokines in this case, we moved to a HRMS instrument (Orbitrap Fusion, Thermo Scientific) with a nanoHPLC (Ultimate 3000, Thermo Scientific) for the chromatographic separation. We evaluated two cytokines: the epidermal growth factor (EGF, 54 amino acids) and the fibroblast growth factor 2 (FGF2, 146 amino acids) with molecular weights of 6348.8 Da and 16397.4 Da respectively. Biological samples (growth medium with and without fetal bovine serum and stem cells) were analyzed both with top down and bottom up approaches. Samples preparation was done following various purification steps, and for bottom up analysis we used trypsin as enzymatic substrate for protein digestion. A PepMap RSLC C18, 2 µm, 100 Å, 75 µm × 50 cm and a PepMap C18, 5 µm × 5 mm, 100 Å. (both from Thermo Scientific) were used as separation and preconcentration columns. Eluents were 0.1% formic acid in water and in acetonitrile (flow 300 nL min^{-1}) for RSLC column, and 0.05% trifluoroacetic acid in water:acetonitrile 8:2 (flow 5 µL min^{-1}) for the preconcentration one. Full mass spectra were acquired with 500 k of resolution in a *m/z* range from 300 to 2000; data dependent analysis were picked up with 60 k of resolution in HCD (high collision dissociation) as activation type.
The calibration curves were obtained in 5mM bicarbonate buffer and in growth medium, with and without fetal bovine serum. The LLOQ was 10 µg/L of EGF and FGF2 in all matrices.
Also in this case, the analyzed samples did not show the presence of cytokines over LLOQ. No signal for the compounds was detectable.
In conclusion, the developed methods based on high pressure liquid chromatography and mass spectrometry were suitable to detect traces of compounds defined as impurities for stem cells.

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O1 AS2

NIR HYPERSPECRAL IMAGING AND MULTIVARIATE IMAGE ANALYSIS FOR THE DETERMINATION OF THE RIND PERCENTAGE IN GRATED PARMIGIANO REGGIANO CHEESE

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Commercial products of grated Parmigiano Reggiano cheese should not contain an amount of rind higher than 18% (w/w), as ruled by the Specification of Parmigiano Reggiano cheese.
Currently, single point near infrared (NIR) spectroscopy is successfully employed to perform fast and non-destructive control procedures to verify product compliance in grated cheese samples [1]. However, it has to be considered that grated cheese contains particles derived from both cheese pulp and rind, resulting in a quite heterogeneous food matrix.
For these reasons, in the present study the use of NIR hyperspectral imaging (HSI), which allows to obtain both spectral and spatial information from a sample, has been evaluated as an effective analytical tool in order to improve the control procedures of Parmigiano Reggiano grated cheese.
To this aim, hyperspectral images of grated cheese samples containing varying percentages of rind were acquired in the 900-1700 nm range. After some preliminary image elaboration steps, including standardization, cropping, background removal and erosion, the hyperspectral images were used to calculate calibration models able to predict the amount of rind contained in the imaged samples. More in detail, the whole dataset of hyperspectral images has been converted into a matrix of signals, namely the *hyperspectrograms*, each one acting like a fingerprint able to codify for the relevant spatial and spectral information contained in the corresponding original image [2, 3]. Then, the hyperspectrogram matrix was used to calculate the calibration models by means of Partial Least Squares (PLS) algorithm.
The results obtained with the proposed approach were compared with the current control procedures based on single point NIR spectroscopy, reaching a 20% decrease of the prediction error.

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O2 AS2

NANOPARTICLE ENHANCED LASER ABLATION INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (NELA-ICPMS)

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A sensitivity enhancement methodology for LA-ICPMS based on the surface plasmon resonance phenomenon as a result of metallic nanoparticles (NPs) deposited on the surface of the sample is proposed. Results show that Nanoparticle Enhanced LA-ICPMS (NELA-ICP-MS) increases the sensitivity up to 1 order of magnitude with respect to the conventional LA-ICPMS without any changes of the experimental set up (i.e. laser parameters or gas carrier composition).
Different kind of metallic nanoparticles (AuNPs, AgNPs, PtNPs) and substrates (metallic and dielectric -Cu, Cu-based alloys, Ti, glass, Si-) were tested.
The enhancement depends on both dropped nanoparticles (kind, concentration and size) and sample tested (investigated element and matrix). Metallic elements show enhancement in both conductive and dielectric matrices, although the better results are obtained on conductive matrix. Different elements show different enhancement in the same matrix, as well as the same element shows different enhancement in different matrices.
Differences in morphology and depth of the craters produced by the laser pulse in the presence and in the absence of NPs, as well as the different size and composition of laser-generated particles allow to attribute to a different laser-substrate interaction the observed enhancement.
In particular, NPs induce locally more efficient ablation below the ablation threshold, that leads to the formation of smaller laser-generated particles, consisting of target material aggregated around NPs, that exhibit better transport/vaporization efficiency, thus enhancing signals for metallic samples.
NPs do not contaminate the sample irreversibly because, after a very limited number of laser shots, they are completely removed from the sample surface.

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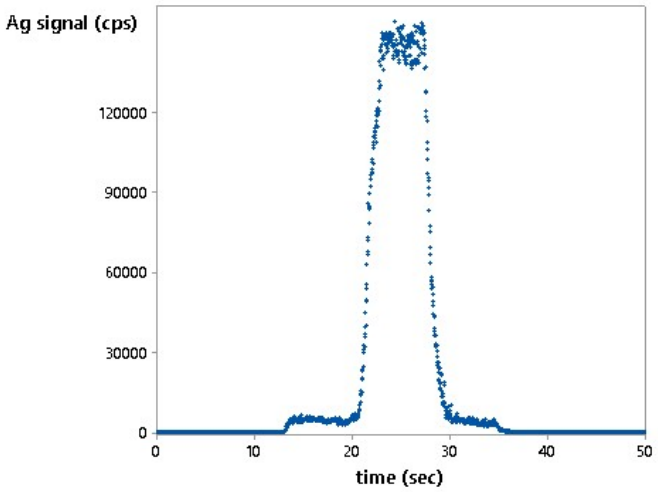

Figure 1. Line analysis of Ag on pure Copper with a 100 µm diameter circular beam at 500 µm/s ablation rate. Laser ablation started 2 mm before (13-20 sec) and stopping 2 mm after (30-35 sec) NPs drop (20-30 sec). PtNPs 17 nm sized, [PtNPs] = 66 fmol/ mm^2

The method developed allows to obtain the same intensity signal as traditional LA-ICPMS by strongly reducing the number of laser pulses on samples, making the technique more suitable for analyses in which negligible destructivity and/or determination of surface-distribution patterns of very thin layers without underlying contamination are demanded. Moreover, it can be particularly useful to cut down isobaric interference (i.e. Cr and Mn interfered by ArO and ArN) because it allow to increase the analyte signal without increasing the interferences, so increasing signal to noise ratio.
The undoubted strength of this approach is represented by its simplicity, affordability and fast performance.

O3 AS2

SURFACE AND INTERFACES CHARACTERIZATION BY XPS OF THE LAYERED GC/Be/Pt ELECTRODE DEVELOPED AS ELECTROCHEMICAL SENSOR FOR BIOMOLECULES DETECTION

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In a previous communication [1] a novel procedure for preparing the composite electrode GC/Be/Pt, showing globular Pt meso-nanoparticles on the outer surface, was presented, based on two consecutive steps:

- as a first step, the betaine film is electrodeposited on GC by cyclic voltammetry (CV) or by pulse electrodeposition technique in a neutral solution containing Be 1,5 mM, giving the modified electrode, GC/Be
- the second one involves a 'controlled' electrodeposition of Na_2PtCl_6 2 mM onto GC/Be by voltammetric procedures, to achieve the finite GC/Be/Pt electrode

The effects of several experimental conditions, the strategic importance of betaine for a better modulation of platinum deposition and related surface morphologies, monitored by Scanning Electron Microscopy (SEM), were critically evaluated also in the light of literature data and properly considered for the use of GC/Be/Pt electrode as electrochemical sensor of important biomolecules such as B- group vitamins [1]

In this contribution based on XPS, the chemical analysis of the outer and intermediate surfaces of GC/Be/Pt electrode was sought to complete its characterization, by individuating the functional groups, responsible of physicochemical interactions, having significance either for interlayer assembly and for sensing biomolecules.

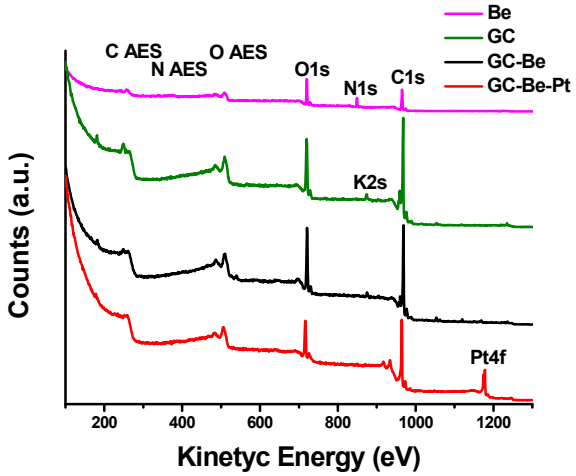

Figure 1. Labelled XPS wide spectra of powdered Betaine (Be) and Glassy Carbon (GC), GC/Be and GC/Be/Pt electrode surfaces

O3 AS2

XPS spectra were acquired with a SPECS Phoibos 100-MCD5 spectrometer, using achromatic MgKα radiation (1253.6 eV). The wide spectra labelling in Figure 1 indicates the elements composing each surface, whose detailed regions were to be acquired at higher resolution and be further resolved by curve-fitting using a well-established program, Googly [2]. The curve-fitted figures of each selected detailed region, have all been plotted with peak assignments (binding energies, BEs) and normalized areas [3], referenced to C1s aromatic carbon, as an internal standard, set at 284.6 eV, and to NIST XPS online database: http://www.nist.gov/srd/surface.htmtd.

These comparative XPS results seem to provide, from both qualitative and semi-quantitative point of view, important indications for the successful preparation of GC/Be/Pt electrodes and for understanding their performance as 'chemical sensors'.

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O4 AS2

MULTI METAL-NANOPARTICLES FUNCTIONALIZED OPTICAL POLYMERIC PLATFORMS AS NEW USEFUL ANALYTICAL TOOL FOR FOOD ANALYSIS

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Nanomaterials (NMs) have become the protagonists of the analytical sciences more and more during the last twenty years, thanks to their unique and modulable chemical and physical properties, giving rise to a plethora of new devices and analytical strategies [1]. On the other hand, the functionalization of 'analytical supports' by polymers (natural or synthetic) represents a useful strategy to obtain active surfaces able to support NMs. Indeed, the matching between the 'surfaces chemistry' and the nanoscience has become a unique opportunity to develop NMs-polymer functionalized platforms, able to solve problems and offer solutions in the analytical field (as e.g. in sensing, biosensing, bioanalysis, catalysis, bioremediation, etc.) [2]. In particular, this multi-disciplinary approach results particular useful and effective for optical supports modification [3]. In this work, a microplate-optical platform, with a polydopamine (PDA) coating functionalized with nanoparticles of different metals, is proposed. This platform has been employed to analyze food constituents as antioxidants and other bioactive compounds. Figure 1 schematizes the platform construction and the general analytical strategy employed. The first step of the microplate-optical platform realization is represented (Fig. 1a), in all the cases, by the self-polymerization of dopamine (DA), resulting in a thin film formation of PDA onto the microplate wells surfaces. Afterward (Fig 1b-e), the residual endogenous catecholamine functions of the PDA thin film, is exploited for the metal (gold and silver) nano-seeds (MSDs) formation, allowing the obtaining of a nanometal-seeds functionalized platform (PDA@MSDs). Fig 1.b-e schematizes the different platforms obtained, that are: mono-metal platforms (PDA@AuSDs and PDA@AgSDs; Fig. 1b), multi-metal platforms (PDA@Au@AgSDs and PDA@Ag@AuSDs) with stratified morphologies (Fig. 1d-e) and core-shell structures (Fig. 1c). For all the supports realized, the PDA polymerization step and the metal seeding have been carefully optimized and characterized by microscopy (SEM) and UV-Vis-spectroscopy. In all the applications implemented, the analytical detection strategy is based on the Localized Surface Plasmon Resonance (LSPR) signal change, related to the MNPs growth or decreases, caused by different analyte concentrations. The specificity and selectivity provided by different analytes is attained thanks to the unique MNPs chemistry and modulated by the different arrangements of the PDA@MNPs platforms. In particular, among the realized platforms, the PDA@AuSDs has been successfully employed to assess the antioxidant power of foods polyphenols, thanks to the seed-mediated AuNPs growth. In this case, the analytical signal recorded is related to the LSPR maximum increase, caused by the

growth of gold seeds driven by the reducing compounds of the sample. The preliminary results obtained seem to be extremely encouraging in terms of antioxidants capacity assessment, for both antioxidants compounds and food extracts. Moreover, works are in progress on the analysis of different food constituents through the use of the other realized PDA@MSDs platforms. Definitely, this work represents the starting point for the realization of an easy to use multi-metal platform aimed to return a food-fingerprinting, able to give information on the whole bioactive compounds pattern.

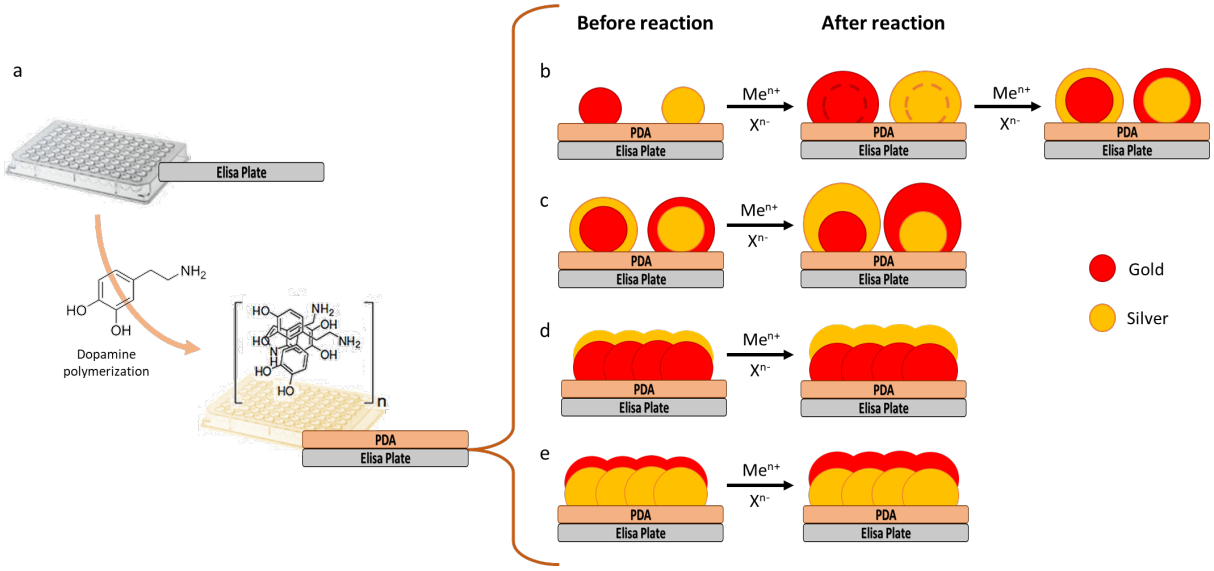

Figure 1. a) DA polymerization on Elisa Plate; b) mono-metal PDA based platform; c) core-shell multi-metal PDA based platform; d) stratified multi-metal (Au@AgSDs) PDA based platform; e) stratified multi-metal (Ag@AuSDs) PDA based platform before and after seeds growth.

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O5 AS2

EFFECT OF CONCENTRATION AND IONIC STRENGTH ON LEAD REMOVAL FROM AQUEOUS SOLUTIONS BY CALCIUM CARBONATE POWDERS

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The removal of toxic elements from contaminated waters is still challenging and innovative remedies are needed. The primary source of these elements is industrial wastewater. Different mechanisms such as adsorption and/or precipitation might be involved in pollutants' removal [1] and novel non-conventional removing methods exploiting industrial byproducts, natural materials, agricultural wastes [2] are investigated in order to comply with the principles of circular economy. In spite of large number of papers concerning these materials, few of them aim to understand the phenomena occurring at the interface between sorbent surfaces and polluted waters. The present work focuses on clarifying the mechanism of interaction between calcium carbonate powders ($CaCO_3$) and Pb^{2+}-model solutions aiming to exploit the wastes from limestone and marble quarries as a resource. The Pb^{2+} concentrations were selected to simulate the content of this toxic element in industrial wastewaters. Advanced surface spectroscopy techniques turn out to be crucial to scrutinize the reactions taking place at the solid-liquid interfaces. X-ray photoelectron spectroscopy was thus used for the chemical state identification of lead and for characterizing the carbonate surface composition resulting from the contact with lead-containing solutions. In order to determine the concentration of lead in solution before and after contact with $CaCO_3$ powders flame atomic absorption spectroscopy (FAAS) was used. The effects of initial Pb^{2+} concentration and ionic strength on Pb^{2+} sorption were examined. Concerning to the toxic element initial concentration effect a wide range of lead concentrations was investigated (from 0.5 μM to 80 mM) to mimic different kind of industrial wastewaters. These solutions were put into contact with commercial $CaCO_3$ 0.1 M; the suspension was stirred for 24 hours at ambient temperature. $NaNO_3$ was added to investigate the effect of ionic strength and the experiments were conducted with an ionic strength of $3x10^{-5}$ M (μ solely due to Pb^{2+} and NO_3^- ions in solution), 0.001 M, 0.01M and 0.1 M while lead concentration was maintained at 0.01 mM (2 ppm). This concentration is way over the Italian legal limit for discharging in surface water and sewerage (0.2 and 0.3 ppm) [3]. These solutions were then put into contact with 0.1 M $CaCO_3$ solution and were stirred for 24 hours at ambient temperature. XPS results showed the presence of Pb on the surface of the $CaCO_3$ samples after contact with Pb^{2+} solutions (Fig. 1 left panel). The high-resolution spectra of the most intense photoelectron signal of lead, Pb $4f_{7/2}$, only showed a single component at 138.8 ± 0.1 eV (Fig. 1 right panel), that might be assigned to $PbCO_3$. Figure 2 (in blue) presents the atomic percentage of lead at the surface of the grains vs the concentration of cation in the solutions. The lead content at the calcite surface increases

with its concentration in the solution. At Pb^{2+}-concentrations lower than 0.01 mM there is a deviation from the linear trend and this might support that the mechanism at low concentrations is different from the one at high lead concentration.

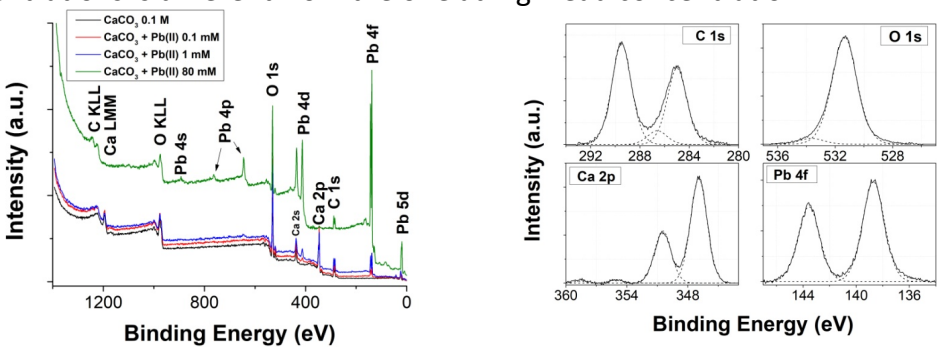

Figure 1 . Survey scans on $CaCO_3$ following contact with solutions containing Pb^{2+} (left image) and high-resolution spectra of C1s, O1s, Ca2p and Pb4f signals acquired on $CaCO_3$ samples following contact with a Pb^{2+} solution 0.05 mM (right image). These latter are shown after subtraction of satellites and curve fitting. Internal reference: adventitious C1s at 285.0 eV.

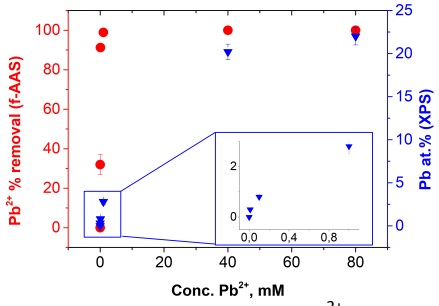

Figure 2. Pb surface concentration (at%) from XPS data vs Pb^{2+} concentration in the solution (in blue). Percentage of Pb^{2+} removed by $CaCO_3$ as a function of Pb^{2+} initial concentration (in red) using FAAS.

Figure 2 (in red) shows the trend for the removal percentage as a function of initial metal concentration, it can be seen a maximum is reached up to Pb^{2+} 1 mM. Preliminary results on the effect of ionic strength on removal processes indicate an increase in removal percentage and a slight increase in sorption capacity in correspondence with the rise of electrolyte concentration. It must be said that this effect could be due to the inhibition of ionization interference in flame atomization processes causing a rise in absorption. XPS data on filtered powders after contact with 0.01 mM Pb^{2+} solution by varying electrolyte concentration showed no observable trend in atomic percentage of Pb on particles' surface. Further investigations are planned for shedding light on the influence of this parameter on lead-sorption on carbonate powders.

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O6 AS2

ELECTRODECORATION AND ANALYTICAL CHARACTERIZATION OF IRON OXIDE NANOPARTICLES WITH BIOACTIVE NANOPHASES FOR TARGETED ANTIMICROBIAL MATERIALS

N. Ditaranto[1], M. Salvador[2,3], A. Moyano[4], V. Marchianò[1], M. Sportella[1], J.C. Martínez-García[2], M. Rivas[2], M.C. Blanco-López[4], N. Cioffi[1]

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Magnetic iron oxide nanoparticles Fe_3O_4 (MNPs) are well known in the oncology field, being used for diagnostic and therapeutic purposes. Indeed, their superparamagnetic properties are exploited to cause the death of cancer cells due to hyperthermia [1,2].
In our work, aiming at exploiting the characteristics of MNPs in a synergistic way with the antibacterial action provided by copper nanoparticles (CuNPs), we have investigated the electrodecoration of iron oxide MNPs by CuNPs [3].
All the magnetic nanoparticles were synthetized via co-precipitation of Fe^{2+} and Fe^{3+} salts in aqueous media, either naked or capped in-situ by polyacrylic acid (PAA) or polyethylenimine (PEI) [4,5]. The Sacrificial Anode Electrolysis (SAE) method [6] has been used to electrodecorate these MNPs in an electrochemical cell, using tetrabutyl ammonium chloride (TBAC) or benzyl dimethyl hexadecyl ammonium chloride (BDHAC) as electrolytes.
All the nanomaterials were characterized by UV-visible Spectrophotometry, Transmission Electron Microscopy (TEM) and X-ray Photoelectron Spectroscopy (XPS).
Magnetite nanoparticles Fe_3O_4 are a mixture of ferric and ferrous anions in a 2:1 ratio. A detailed surface chemical investigation was performed to identify the Fe^{2+} and Fe^{3+} features present in the XPS peaks. Since the main XP photoelectron Fe2p peak is well known to be a very complex system, a combined study of both secondary peaks (Fe3p) and valence band region (VB) was performed. In particular, the VB shape is considered a kind of "fingerprint" of magnetite composition. Moreover, in many studies the peak position of $Fe2p_{3/2}$ with respect to the satellite peak [7] along with the shape of valence band [8] are accounted for differentiating Fe oxidation state, that is ferrous and ferric oxide. According to the literature, MNPs low stability in air led to maghemite (γ-Fe_2O_3) formation, ending up with a Fe_3O_4-Fe_2O_3 core−shell structure.
Moreover, surface spectroscopy and morphological analyses demonstrated that interactions between the different nanophases occurred in composite materials, since the resulting nanomaterials both retain the MNPs magnetic properties and reveal new spectroscopic features in the valence band region of modified magnetite compared to the bare sample (Figs. 1a, 1b).

O6 AS2

UV-vis and magnetic measurements are in progress, as well as the assessment of the antibacterial effects of the nanocomposite materials.

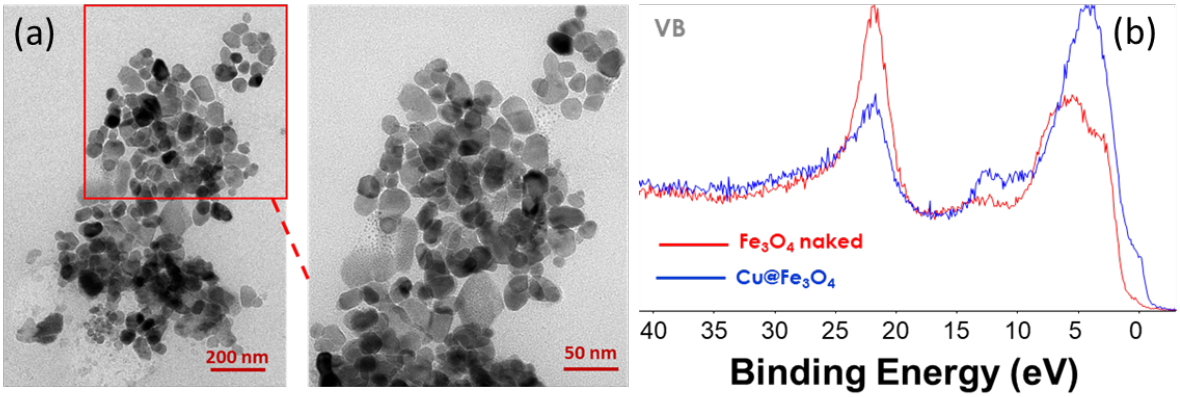

Figure 1. TEM images at different magnifications of BDHAC stabilised CuNPs@Fe_3O_4 (a) and XP valence band spectra comparison of naked and CuNPs@Fe_3O_4 samples (b).

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Poster presentations

P1

URINARY GC-MS UNTARGETED STEROIDOMICS FOR THE PREDICTION OF BUBBLING RISK IN DIVERS. PRELIMINARY RESULTS

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The production of nitrogen bubbles in the blood during a dive is a phenomenon occurring frequently and a risk factor for endothelial dysfunctions [1]. Thus, predictive tools are necessary for ensuring the safety of the divers.
Metaboloma modifications due to altered environmental conditions are known. With the present study, we wanted to investigate the occurrence of *a priori* metabolic differences between non-at- and at-risk divers.
The urines from 19 experienced divers were sampled during an indoor training. They were classified, following the Eftedal and Brubakk scale, as *bubblers* (n = 7), *non bubblers* (n = 9) and *occasional bubblers* (n = 3). The samples were pre-treated using an optimized procedure and derivatized with trimethylsilyl mixture, and subsequently analyzed using a gas chromatography-mass spectrometry (GC-MS) method. The full-SCAN chromatograms were aligned using the correlation optimized warping (COW) along both the time and m/z dimensions [2]. Then, the PARADISe software, based on the PARAFAC2 algorithm [3–5], was used for peak deconvolution and features extraction. A total of 131 compounds were obtained, and the corresponding MS spectra were compared with the NIST libraries. The 3 samples provided by the occasional bubblers were initially discarded from the dataset and the remaining 16 profiles were used to build a bubblers *vs* non-bubblers classification model. The variables selection, involving Variable Importance Projection and Genetic Algorithms, was performed before the Partial Least Square–Discriminant Analysis [6]; a total of 25 variables were selected, and most of them belong to the biological class of steroids. The robustness of the classification model was tested using a repeated double Cross-Validation strategy with the leave-one-out approach [7], which resulted in zero misclassified subjects. Finally, the occasional bubblers were projected onto the model: their scores values fell between the ones of the two populations (see Figure 1), and the model predicted them as bubblers, guaranteeing a precautionary approach.

P1

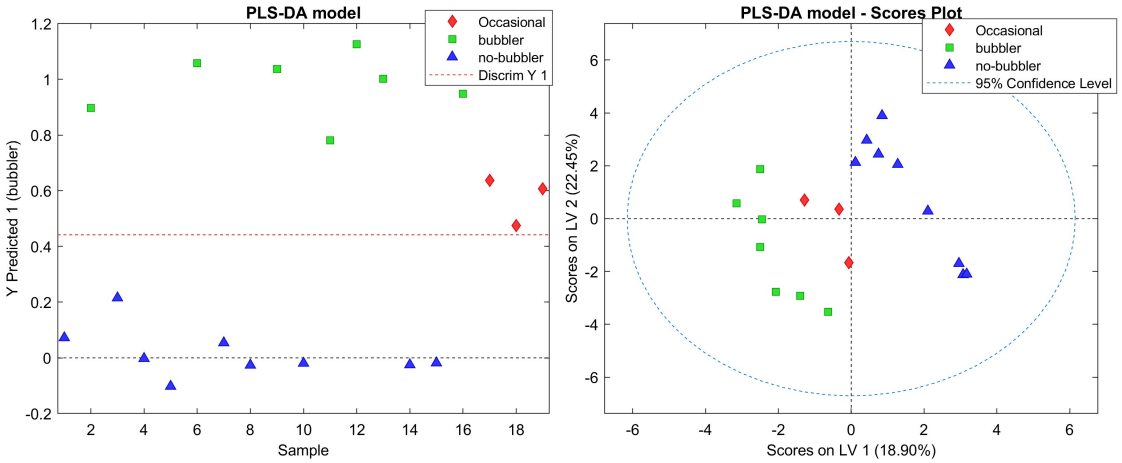

Figure 1. Bubblers vs no-bubblers PLS-DA classification model. The red diamonds correspond to the occasional bubblers, classified by the model as bubblers.

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P2

CHARACTERIZATION OF GLYCOSPHINGOLIPIDS IN LUPINUS LUTEUS BEANS BY REVERSE PHASE LIQUID CHROMATOGRAPHY COUPLED WITH ESI-MS

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Lupin beans can be considered as a nutraceutical food because they are known to reduce the glycemic index and to play a key role in opposing obesity and illnesses such as diabetes and heart diseases [1]. In order to correlate these beneficial properties to the composition of yellow legume seeds, many studies have been carried out mainly on the characterization of protein content [2], vitamins [3] and lipids [4]. There are very few studies focusing on the complex lipid content in these seeds [5] while the complete characterization of lipid classes is absent. A novel strategy to describe the lipidome of *L. leutus* seeds, based on hydrophilic interaction liquid chromatography (HILIC) and mass spectrometry, is proposed here. Although more than 200 major phospholipids (PL) were identified by HILIC-ESI-FTMS, we focused here on two minor lipid classes: ceramides (Cer) and hexosylceramides (HexCer) never described before in these edible beans. Sphingolipids (SL) and glycosphingolipids (GSL), in plants, participate in different biological processes, such as cell cycle, apoptosis, response to hypoxia and pathogen attack [6]. GLS are glycolipids containing a ceramide with two hydrophobic chains (one amine-containing lipid referred to as the sphingoid base and one fatty acid tail) and a glycan head moiety (oligosaccharide). Since the elution time of these two minor lipid classes is close to dead volume in HILIC, a reverse-phased liquid chromatography (RPLC) separation was introduced along with a specific extraction protocol [7]. The RPLC separation was coupled with electrospray ionization in negative ion mode (ESI(-)) and multistage tandem mass spectrometry, MS/MS and MS^3, using a linear ion trap (LT Velos Pro). The identification of SL and GSL was attained by accurate *m/z* values recovered under the RPLC bands and used as input values of an online database (Online lipid calculator); tandem MS spectra acquired by collisional-induced dissociation (CID) afford information on the type and saccharide number and ceramide moiety (i.e., N-acyl residue and long-chain base) while CID-MS^3 spectra on the ceramide anions help the sphingoid base assignment [8][9]. As an example, in Figure 1, the RPLC-ESI(-)-MS/MS spectrum of the precursor ion at *m/z* 694.6718 is shown, for which the database suggests a Cer 44:0;3 as a deprotonate molecule. In the same figure it is possible to see signals at *m/z* 676.6 and 658.6 associated with one or two losses of water molecules from precursor ion, at *m/z* 395.3 corresponding to fatty acid 26:0, and *m/z* 394.3 corresponding to the same with the amino group. The signal at *m/z* 420.3 represents the fragment $[Cer\text{-}H\text{-}274]^-$, while the ion at *m/z* 450.3 is generated from the fatty acid and a portion of the sphingoid base. The *m/z* 267.2 ion is a diagnostic fragment of phytosphingosine; it can be concluded that the species under investigation can be assigned as Cer t18:0/26:0. The deep inspection of the MS/MS spectrum

showed the occurrence of an isobaric species, Cer t20:0/24:0. By following this approach, 25 Cer and 43 HexCer were identified in *L. leutus* seeds in terms of sugar moiety, sphingoid base and fatty acid chain.

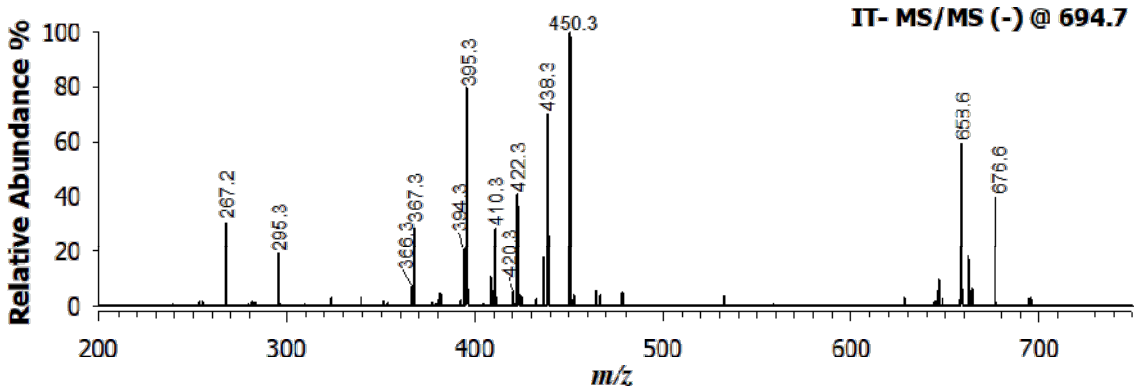

Figure 1. RPLC-ESI(-)MS/MS spectrum of the deprotonated molecule at *m/z* 694.7.

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P3

LC-ESI-MS AND TANDEM MS ANALYSIS OF SULFOQUINOVOSYL MONO- AND DIACYLGLYCEROLS IN EDIBLE ALGAE EXTRACTS

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Aquatic algae are plant organisms that populate seas, rivers, lakes and ponds around the world, often living on rocks and on damp soils; these species have been the subject of numerous studies of both biological and chemical interest even as biodiesel fuel [1]. Tasty and rich in benefits [2][3], algae have been consumed since ancient times. Currently, there is a great demand for healthy and natural foods and edible algae represent an interesting answer. They are nowadays proposed as supplements to our daily diet or alternatively as an excellent ingredient for soups and salads, to which they add a good low-calories protein value. They are considered as a healthy food since they are toned, emollient, anti-infective and antioxidant, rich in minerals, vitamins, fiber, lipids and proteins. The consumption of algae helps the absorption of vegetable omega 3 fatty acids, which is very important for people who do not eat fish [4]. However, algae are not rich in lipids and their chemical characterization has not been addressed systematically.

The main purpose of our work was to describe the lipid profile of edible algae; here we report the detailed characterization of a class of glycolipids containing sulfur known as sulfoquinovosyldiglycerides (SQDG) together with their lyso-forms SQMG [5]. Specifically, four edibles dried macroalgae were analyzed: Nori (*Porphyra spp*.), Wakame (*Undaria pinnatifida*), Dulse (*Palmaria palmata*), Kombu (*Saccharina japonica*) and a microalga Spirulina (*Arthrospira platensis*). Lipid fraction was extracted from sample using a Bligh & Dyer protocol [6]; after a purification on a microcolumn by solid phase extraction (SPE), lipids were analyzed by reverse phase liquid chromatography (RPLC) and electrospray ionization (ESI) coupled to high resolution mass spectrometry (MS) (Figure 1). Thanks to the presence of sulphonic group these species are easily deprotonable so the acquisition of mass spectra (MS) and tandem mass spectra (MS/MS) was performed in negative ion mode.

A first identification of the lipid class was attempted by submitting the accurate *m/z* values (less than 5 ppm) to an online database (*Online Lipid Calculator*) that reports in output the putative lipid class together with the total number of carbon atoms and unsaturations. Each assigned *m/z* value to SQDG and/or SQMG was isolated as precursor ion and subjected to fragmentation by HCD-MS/MS experiments; the resulting spectra were interpreted following rules the established earlier [7].

P3

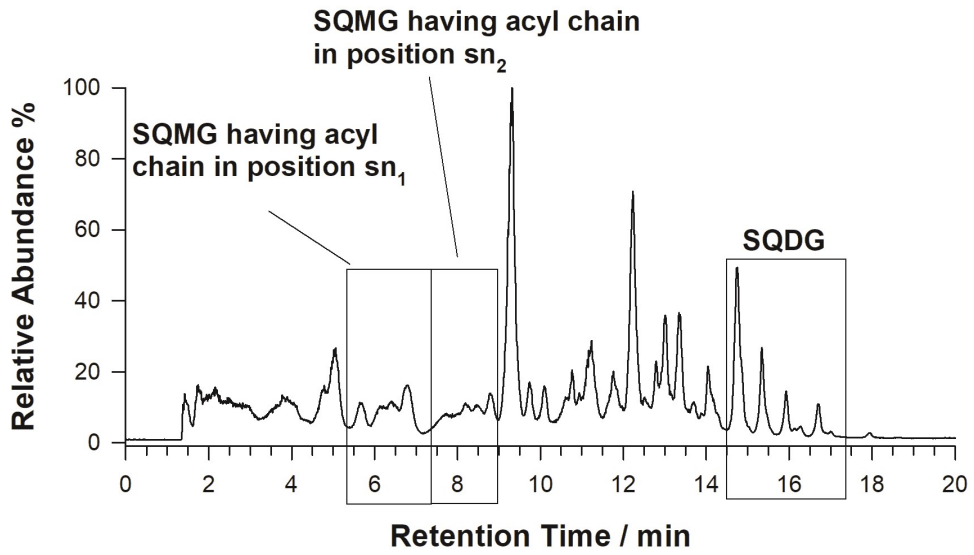

Figure 1. Chromatographic profile by RPLC-ESI(-)FTMS of a sample extract of Wakame seaweed.

Starting with the lipid extract of Wakame seaweed samples, 16 more abundant SQDGs and 6 SQMG (i.e., lyso-forms) were characterized and their regiochemistry assigned. A comparison will be presented and discussed with all four investigated algae extracts highlighting the most relevant difference both from qualitative (i.e., chain length, number of double bonds and sn_1/sn^2 position) and quantitative (relative contents) point of views of SQMG and SQDG.

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P4

VERTEBRATE ODORANT BINDING PROTEIN: BACTERIOSTATIC AND FUNGISTATIC AGENT AGAINST PATHOGENIC MICROORGANISMS

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Antibiotic-resistant bacteria are one of the major threat to public health, especially for hospitalized patients. Lung infections are responsible for most of the morbidity and mortality in patients affected by cystic fibrosis. In recent years, the antibiotic abuse resulted in the emerging and worldwide diffusion of multidrug-resistant bacteria and fungi [1].
Quorum sensing (QS) is the mechanism used by bacteria and fungi to coordinate several biological functions, including formation of biofilm formation, immune response, motility and production of virulence agents [2]. This cell-to-cell bacterial communication relies on the production, secretion and detection of small diffusible signaling molecules called autoinducers (AIs). The disruption of these signals, known as quorum quenching approach (QQ), requires the use of chemical agents able to complex or degrade the AIs or inhibit their binding sites. QQ-based therapies could reduce the side effects compared to common treatments and overcome the antibiotic resistance of pathologic microorganisms. Several classes of QQ agents, exerting different activity against diverse pathogens, are under investigation. Odorant binding proteins (OBP) are multifunctional protein carriers dissolved at millimolar levels in the mucus layering the epithelia of the respiratory apparatus of vertebrates [3]. Belonging to the lipocalin family, these proteins present an internal cavity layered by non-polar amino acid residues, i.e. the ligand binding site able to accommodate a large variety of hydrophobic structurally unrelated organic compounds with molecular masses in the 150-350 Da range [4]. Previous studies dealing with the bovine and porcine forms of OBP (bOBP and pOBP, respectively), demonstrated the binding activity toward compounds structurally related to farnesol and N-acyl-homoserine lactones (AHLs) [4], two of the most common and well-recognized classes of AIs.
In a research framework dealing with the development of nanocarriers based on the use of OBPs as QQ agents for the treatment of multidrug-resistance microorganisms [5], the aim of this study is to demonstrate the bacteriostatic and fungistatic activity of OBPs and synthetize and characterize a hybrid nanoparticles system characterized by superparamagnetic iron oxide core and functionalized with odorant binding proteins. In order to investigate the OBP ability to interfere with virulence and cross-communication of pathogenic microorganisms, ligand binding assays were performed to test the scavenging potential of bOBP and pOBP toward farnesol, AHL, oxo-AHL and pyocyanin. In addition, the direct antimicrobial activity of the OBPs was tested by time kill assay (TKA) against several bacteria (with particular

attention paid toward *Pseudomonas aeruginosa* - PA) and yeasts like *Candida albicans* (CA). The positivity of all the ligand binding assays and the antimicrobial activity, demonstrated for several microorganisms, could be considered as a first step to prove that vertebrate OBPs behave as humoral components of innate immunity, active against pathogenic bacteria and fungi. Furthermore, the 6 tagged histidine mutant form of bOBP (6 His-tag-bOBP) proved to be characterized by enhanced interfering activity against different pathogenic microorganisms, including PA and CA [5].

Based on these results, a hybrid system presenting a superparamagnetic iron oxide core functionalized with the 6 His-tag-bOBP was developed. The nanoparticles (NPs) were designed in order to allow the magnetic protein delivery to inflamed areas of lungs. The use of hybrid biosystems could improve the targeting toward a specific region of lungs by using an external magnetic field, thus increasing the local drug concentration and reducing the side effects. The synthesis procedure is schematized in Figure 1.

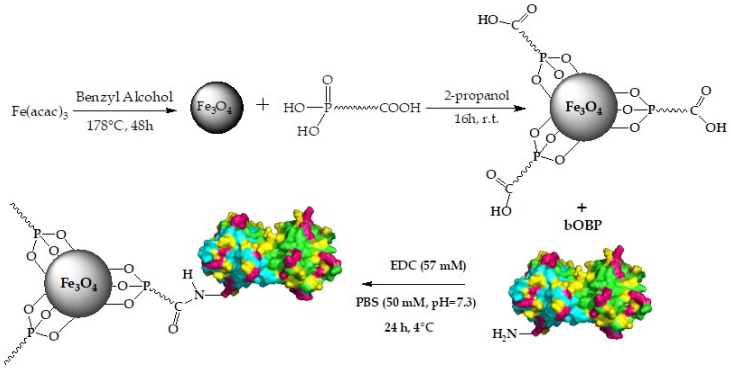

Figure 1. Synthesis procedure of bOBP-functionalized SPIONs

The inorganic superparamagnetic core was characterized by a spherical shape with an average diameter of 6.5±1.1 nm. 11-Phosphonoundecanoic acid was used as spacer and the NPs were linked with bOBP *via* amide bond using EDC as coupling agent under mild conditions in order to keep the protein folding unaltered. The concentration of the loaded bOBP, measured by commercially available protein assay kit, was 26.0±1.2 mg bOBP/g SPIONs. Finally, the QQ activity of the system was tested *in vitro* against CA and PA.

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P5

DETERMINATION BY GC-MS OF ORGANIC POLLUTANTS IN ETNA VOLCANIC ASHES

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Mount Etna, define as UNESCO heritage since June 2013, is one of the most active continental volcanoes. In the last decade, volcanic eruptions has been produced huge amounts of gases and solid particles impacting the life with a number of effects. Volcanic ash travels hundreds of kilometres downwind from the volcano generating the characteristic ash falls. The ash fall causes several complication to human life and to economic activity by covering road and airport runways with expensive disposal activity. Etna volcanic ashes are classified as waste with EWC 170504 (soil and stones) and destined for recovery operation.
According to Directive 2008/98/EC "end-of-waste", this study aims to evaluate a possible role of volcanic ash as scavenger of organic pollutants such as polycyclic aromatic hydrocarbons (PAHs)[1].
PAHs are well-known organic pollutants and they may occur either from natural sources (e.g. volcanic eruptions) or anthropogenic activities [2].
For this purpose a preliminary characterization of Etna volcanic ashes was performed. We developed an analytical method for the quantification of 19 PAHs in volcanic ashes using GC-MS. Spiking experiments at 5 μg/Kg provided recovery range from 70 to 120% for all compounds. Preliminary data on Etna ashes, sampled at different altitude, show the presence of the three- to six-ring PAHs, suggesting a mixture of petrogenic and pyrogenic sources. Based on these evidence, Etna volcanic ashes could be employed as natural support in catalytic processes for removal of organic pollutants.

Progetto "Recupero e utilizzo delle ceneri vulcaniche etnee (REUCET), University of Catania"

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P6

DEVELOPMENT OF AN ANALYTICAL STRATEGY FOR THE METAPROTEOMIC CHARACTERIZATION OF ATMOSPHERIC BIOAEROSOL IN WORKING ENVIROMENTAL SAMPLE

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Metaproteomic analysis of air particulate provides information about the properties of bioaerosols in the atmosphere and their influence on climate and public health. In this work, a new method for the extraction and analysis of proteins in airborne particulate matter from quartz microfiber filters was developed. In particular, five different extraction protocols were compared to select the best extraction procedure for an effective metaproteomic analysis for proteins within cells, free proteins and proteins bound to the aerosol material. The five extraction procedure used 50 mmol L-1 tris-HCl (pH 8.8) with protease inhibitors and added with A) sodium dodecyl sulfate (SDS), ethylenediaminetetraacetic acid (EDTA) and 1,4-dithiothreitol; B) glycine and SDS; glass beads with C) SDS; D) sodium deoxycholate; E) sodium dodecanoate. Initially, methods were compared through the recovery of free proteins, which was determined by spiking 5 different standard proteins on blank filters: BSA, CYT-C, ApoMb, ApoTF and IgG were chosen as representative. Protocols A-C required a precipitation step to remove SDS and EDTA and resulted not compatible with protein trace analysis. As size exclusion chromatography was previously applied to glass fibre filters [1], a filter aided sample preparation protocol was tested, but it was not suitable due to the presence of carbon in the extract. Protocols D and E exploited detergents compatible with protein digestion and can be precipitated by acidification. Before analysing the samples, further evaluation experiments were performed on protocol D, to simulate a more realistic condition and evaluate the extraction efficiency of proteins within cells; protocol D was applied to blank filter samples spiked with the spores from a ubiquitous bacterium, i.e Bacillus Subtilis. The optimized method was finally applied to the analysis and characterization of filters from different work environmental sites collected in the Lazio (Italy) and influenced by urban and rural boundary layer air masses. The developed method was based on shotgun proteomics: digestion by trypsin, separation by nanoHPLC and analysis by high resolution tandem mass spectrometry and bioinformatics. 179, 15, 205 and 444 proteins were identified in the composting plant, the wastewater treatment plant and the agricultural holding, respectively. In agreement with the major categories of primary biological aerosol particles, all identified proteins were mainly originated from fungi, bacteria and plants. This is the first metaproteomic study applied to bioaerosol samples collected in occupationally relevant environmental sites and, even though not aimed at

monitoring the risk exposure of workers, it provides information on the possible exposure in this sites.

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P7

COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY COUPLED TO TIME OF FLIGHT MASS SPECTROMETRY FEATURING TANDEM IONIZATION: ADDING AN EXTRA-DIMENSIONS TO HAZELNUTS (*Corylus avellana* L.) PRIMARY METABOLOME FINGERPRINTING

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This study focuses on hazelnuts (*Corylus avellana* L.) primary metabolome (i.e., amino acids, mono and disaccharides, low molecular weight acids and amines) and its characteristic fingerprint as a function of geographical origin, harvest year, post-harvest drying and storage time. Its information potential is of great interest to predict hazelnuts sensory quality after industrial roasting. Most of the (key)-aroma compounds and potent odorants [1] derive from non-volatile precursors that, once mapped, may objectively represent nuts potential quality.
Comprehensive two-dimensional gas chromatography coupled to Time of Flight Mass Spectrometry (GC×GC-TOF MS) featuring Tandem Ionization by varying electron energies across the analytical run is exploited and 2D-patterns of derivatized primary metabolites (oximation-sylilation) are explored by combined untargeted/targeted fingerprinting (UT fingerprinting) based on the template matching strategy [2].
The primary metabolome accounts of about 500 2D-peak-regions as illustrated in the color plot of Figure 1; within these detectable analytes, a sub-group of about 150 can be reliably identified by matching linear retention indices (LRI) and MS spectra at 70 eV. Characteristic fingerprints at 70 and 12 eV ionization energy enable both sample clustering on the basis of key-variables (origin and cultivar) and results cross-validation. In addition, soft ionization energy at 12 eV produces spectra with a complementary information power and higher specificity for most of the informative analytes. Spectra at 12 eV have higher relative ratio for heavier fragments and lower intensity for derivatization agents.
By combining data from primary metabolite distribution and volatiles, produced after lab-scale model roasting, several positive correlations (statistically relevant) between precursors and Maillard reaction products confirm the consistency of the proposed approach and the high flexibility of the analytical platform.

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P8

MICROFLUIDIC OPEN INTERFACE WITH LIQUID ELECTRON IONIZATION MASS SPECTROMETRY: RAPID MEASUREMENT OF THC IN DIFFERENT MATRICES

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Delta-9-tetrahydrocannabinol (THC) is a chemical found in Cannabis sativa. THC is the primary active ingredient and responsible for most of marijuana's psychological effects. All legal products based on cannabis have residues of THC not always reported accurately on the product label. We present a novel microfluidic open interface which is able to generate nL flows highly compatible with liquid electron ionization mass spectrometry (MOI-LEI-LC-MS) as a sensitive and universal technique that efficiently integrates sampling and sample preparation steps with a direct introduction to EI-MS, facilitating a fast coupling of DI-SPME devices using a microfluidic design.
MOI consists of a PEEK cylinder (internal volume of approximately 10 µL) functions as the desorption device for SPME C18 fibers. The new MOI design can generate nL/min flows and it is different compared with an originally described microfluidic open interface (MOI), still sharing the same operating principles [1]. It is connected to the nLC pump and LEI interface via a tee and a sixport switching valve. Desorption solvent: 100% acetonitrile (ACN) at 1 µL/min, continually entering the ion source. The estimated liquid volume surrounding the fiber (1 cm x 240 µm) is 1.5 µL. LEI is an ideal pairing with MOI because the gas-phase ionization process produces negligible matrix effects and allows real-time quantitative detection of target compounds without chromatographic separation [2]. The switching valve has two positions: 1. Chamber filling, ACN flows from the pump to MOI and LEI-MS, keeping the desorption chamber full; 2. Static desorption (a)-injection (b): (a) ACN flows from the pump to waste, LEI-MS is isolated, the fiber, after sampling, is introduced into MOI. (b) Sample injection: the fiber is removed after 1 min of desorption, MOI port is closed, and the valve is switched back to position (1). In this position, ACN pushes the desorbed analytes to LEI-MS.
An Agilent 1100 nLC pump is coupled with an Agilent MDS 5975C single quadrupole using a LEI interface. LEI conditions: inlet capillary: 30 µm; vaporization microchannel T 400 °C; source T 300 °C; quadrupole T 150 °C; acquisition mode SIM m/z: 231; 251; 299; 314. MRM experiments have been scheduled to increase sensitivity and selectivity in real samples.
Preliminary studies were performed on THC in water optimizing all the process: times of sampling and of desorption, flow rates, twisting or no twisting the fiber during desorption, position of the fiber into the desorption chamber. Calibration studies: 3 ml of pure water were added of THC at 0.1, 0.5, 1, 2, 5 µg/mL. SPME C18 fiber conditioning: 100% ACN (30 min). Sampling and MOI-LEI-LC-MS: fiber immersion in the sample (1 h); fiber desorption in MOI (1 min). Three consecutive extractions experiments were performed, showing good reproducibility (6.4 RDS%). A limit of detection of 10 ng/mL was estimated.
An infusion of diluted legal Cannabis sativa leaves was analyzed and quantified (1.5 g in 200 mL of boiling water for 10 min).

P8

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P9

OPTIMIZATION BY RESPONSE SURFACE METHODOLOGY OF A DISPERSIVE MAGNETIC SOLID PHASE EXTRACTION EXPLOITING MAGNETIC GRAPHENE NANOCOMPOSITE COUPLED WITH UHPLC-PDA FOR SIMULTANEOUS DETERMINATION OF NEW ORAL ANTICOAGULANTS (NOAs) IN HUMAN PLASMA

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The present study focused on the synthesis of a graphene based magnetic nanocomposite (rG/Fe_3O_4) as an optimal material for MSPE and investigated its performance for adsorption of NOAs in human plasma coupled with UHPLC-PDA determination.
The nanocomposite was characterized using FTIR spectroscopy and AFM measurements to obtain chemical and morphological information about the samples.
The properties rG/Fe_3O_4 as sorbent in dMSPE were investigated and several key parameter including nanocomposite amount, sample pH and extraction time were optimizated to maximize the recovery of the investigated NOAs.
In this work we adopted a response surface methodology (RSM) coupled with box-Behnken design of experiment (DOE) to evaluate the influence of some parameters on the dMSPE efficiency. This multivariate approach allows to obtain reliable results minimizing the experiments compared to the classical one-variable-at-time approach (OVAT). The models F-value for first order, two way interactions and pure quadratic, indicate that the models were significant at $p < 0.05$ for Apixaban, Dabigatran and Rivaroxaban response.
Chromatographic separation was obtained using a kinetex EVO C_{18} (100 x 2.1 mm I.D. 2.6 µm particle size) protected by kinetex EVO C_{18} precolumn.
The mobile phase was a mixture of 10 mM acetate buffer adjusted to pH 3 with acetic acid (phase A) and methanol (phase B). A linear gradient elution program was used for the separation of API, DAB, RIV and the internal standard. At a flow rate of 0.7 mL / min, the total run time was 7 min.

P9

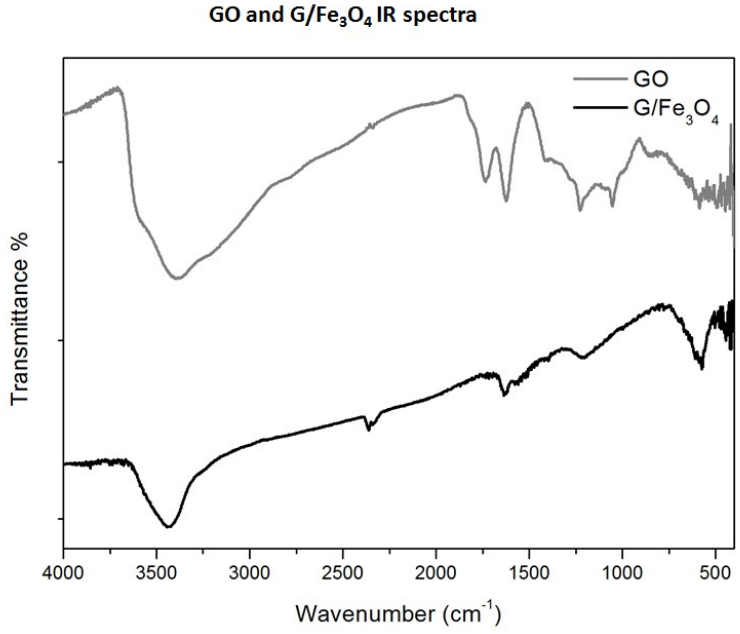

Figure 1. IR-spectra of graphene oxide and the synthesized nanocomposite

The proposed method was validated according to the FDA guidelines in the range of 0.005 – 10 µg/mL, accuracy was within -6.54% and + 4.21 % (BIAS %) while precision was below 3.21% (RSD %).
The main recovery for apixaban, rivaroxaban and dabigatran ranged within 96.6 to 98.9 with a low relative standard deviation (RSD% < 2.34).

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P10

USING LABELLED INTERNAL STANDARDS TO IMPROVE NEEDLE TRAP MICRO-EXTRACTION TECHNIQUE PRIOR TO GAS CHROMATOGRAPHY/MASS SPECTROMETRY

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The amount of water vapour collected in a needle trap along with volatile analytes may vary from sample to sample and decrease during the storage, when working with humid gaseous samples. This has a major impact on desorption efficiency and recovery. In this work, we propose the addition of labelled internal standards to nullify the effect of variable humidity on the analytical performance of needle trap micro-extraction combined with gas chromatography mass spectrometry. Triple-bed (Divinylbenzene/Carbopack X/Carboxen 1000) and single-bed (Tenax GR) needles were tested with standard gaseous mixtures prepared at different relative humidity levels (85%, 50% and 10%). The standard mixtures contained twenty-five analytes representative of breath and ambient air constituents, including hydrocarbons, ketones, aldehydes, aromatics, and sulphurs, in the concentration range 0.1–700 ppbv. The two needles showed different behaviours, as recovery was independent of humidity for single-beds, whereas a low recovery (10–20%) was observed when triple-beds trapped very volatile compounds at low humidity (e.g. pentane and ethanol, 10% relative humidity). Triple-beds showed an almost quantitative recovery (> 90%) of all the analytes at 50% and 85% relative humidity. This big difference was probably due to the reduced action of water vapour pressure during the desorption step. The addition of ^{6}D-acetone and ^{8}D-toluene to the sorbent material before gas sampling and the normalization of raw data nullified this effect, thereby lowering the variations of analyte recovery at different humidity levels down to 20%. Internal standards were also exploited to limit within 10–20% alterations in peak areas of very volatile compounds during needle storage at room temperature. This variation may results from a loss of water vapour either retained from the sorbent material and/or condensed on triple-bed needle walls. After normalization, the inter- and intra-day precision were halved to 5% and 10% in the case of single-beds, respectively, and to 15% and 20% with three-beds. The addition of an internal standard to the sorbent helps to keep the overall analytical procedure under control and improves the reliability of needle trap micro-extraction for the analysis of volatile organic compounds at ultra-trace levels.

P11

ELECTROSTATIC ATTRACTION-REPULSION MODEL WITH *CINCHONA* ALKALOID-BASED ZWITTERIONIC CHIRAL STATIONARY PHASES

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The *Cinchona* alkaloid-based zwitterionic chiral stationary phases (CSPs), based on zwitterionic chiral selectors (SOs) [1-3], were successfully employed for the enantioseparation of underivatized constraint amino acids (the selectands, SAs) even in the absence of ionic additive, generally used as displacer counter-ions into the eluent. This feature can be in principle ascribed to the so-called "intramolecular counter-ion (IMCI)" effect (Fig. 1): the cyclohexanesulfonic acid group of the SO moiety acts as an intramolecular counter-ion for the acidic moiety of the zwitterionic SA, while the quinuclidine site behaves as an intramolecular counter-ion for the cationic site.
In the present study, the IMCI revealed to be sufficient to modulate alone the retention of four β-amino acids selected as target compounds for the study. The analyses were carried out with either neat methanol, acetonitrile, water or their binary hydro-organic mixtures. A U-shaped retention profile was observed both with methanol- and acetonitrile-based eluents. The electrostatically driven attractive SO-SA interactions active in highly organic eluent systems got weaker as the water content in the eluent was increased. This behaviour persisted until a definite hydro-organic eluent composition was reached. After this so-called "balanced region", hydrophobic interactions became significant with water rich mobile phases, thus promoting a new increase of analyte retention. Except few cases, enantioselectivity and enantioresolution were achieved with most of the screened mobile phases. An electrostatically driven "attraction-repulsion (pull-and-push) model" was postulated to explain the very favourable characteristic of the two studied CSPs for the retention and enantiomer separation of zwitterionic analytes. This mechanism was also successfully applied for the additive-free enantioseparation of some rigid α-amino acids.

P11

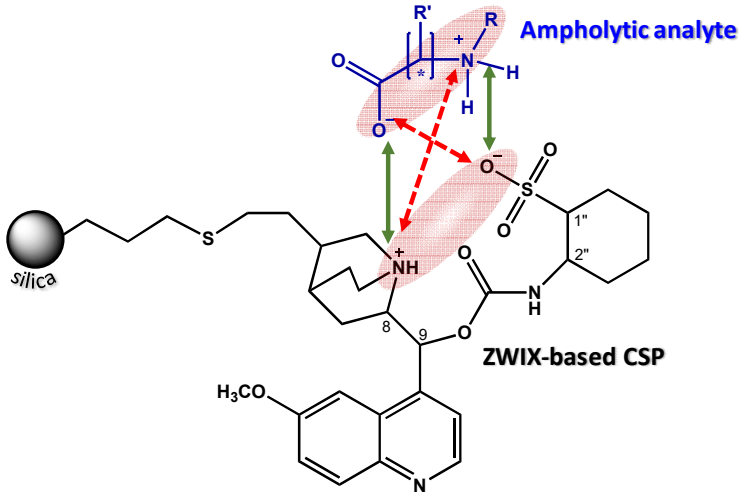

Figure 1. Electrostatic "pull-and-push" model proposed between a ZWIX-based CSP and an ampholytic analyte

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P12

ADVANCED CHARACTERIZATION OF ARCHAEOLOGICAL BEESWAX AND RESINOUS MATERIALS BY HIGH RESOLUTION MASS SPECTROMETRY

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The characterization of archeological adhesives is an important research area in Heritage Science, since allow to define a correlation between the chemical composition of these materials with the technological advancements of mankind.
The most widespread approaches used for the characterization of beeswax and resinous materials are based on wet chemical sample pretreatment and gas chromatography-mass spectrometric analysis (GC/MS). Alternatively, analytical pyrolysis (Py) coupled with GC/MS has been also applied.
These approaches have a critical limitation since a hydrolysis/thermal decomposition step is performed, which could lead to a possible loss of information on the original composition of the organic residues.
In order to obtain an exhaustive picture of the composition of both archaeological beeswax and resinous materials, in this study we propose a fast analytical approach based on flow injection analysis (FIA) in the high-resolution mass spectrometer throw an ESI source (ESI-Q-ToF).
In order to maximize the amount of information that can be obtained on a single archeological sample, the adhesives were extracted using a micro-wave assisted approach, that allow to increase the extraction yield respect to the traditional approaches, avoiding the possible occurrence of transesterification.
This approach was used for the characterization of the original adhesives used to fix monochrome and mosaic glass and stone plaques coming from the archaeological site of Antinoopolis, dated back to the 4th-5th century AD. These materials came from the parietal and floor decorations of the religious buildings and mortuary chapels of the northern necropolis.

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P13

USE OF AN INTELLIGENT SAMPLING DEVICE (I KNIFE), COUPLED TO RAPID EVAPORATIVE IONISATION MASS SPECTROMETRY TECHNOLOGY, FOR AUTHENTICITY ASSESSMENT IN PISTACHIO SAMPLES

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Rapid Evaporative Ionization Mass Spectrometry (REIMS) is an emerging technique that allows rapid characterization of different real-world samples with no need for extraction or cleanup procedures. It is based on a fast evaporation of a sample, yielding gaseous molecular ions of the major components, thus obtaining a holistic profile, potentially usable as univocal fingerprinting.
Since its introduction in 2009 by Professor Zoltan Takats of Imperial College, London, it was mainly used for in vivo biological tissues analysis, since, differently from desorption ionisation methods, it does not require any sample preparation. In 2013, Takats and co-workers coined the term "intelligent knife" (iknife) to indicate the coupling of REIMS with an electrosurgical knife. The iknife tool creates a smoke rich of biological information that can aid the surgeon in real time to decision-making during surgery.
The aim of the present research is to extend the applicability and the advantages of such a technique to food samples for geography evaluation and authenticity assessment. In order to achieve this object, a database containing MS profiles for authentic available samples need to be created.
Within this context the application here described focus on the profiling of pistachio samples, taking into account that adulterated sicilian Bronte pistachio can be found on the market, due to its high cost. The generation of a specific MS profile for Bronte pistachio nuts by the novel iknife technology will be very helpful to unambiguously assess its authenticity.

P14

RAPID AND RELIABLE IDENTIFICATION OF SEMI-VOLATILE COMPOUNDS BY SUPERCRITICAL FLUID CHROMATOGRAPHY COUPLED TO ELECTRON IONIZATION MASS SPECTROMETRY DETECTION

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Supercritical fluid chromatography (SFC) is a substantially new technique, whose rapid spread and increasing interest are mainly related to aspects of eco-compatibility, low cost of analysis and high analytical performance. Furthermore, the possibility of coupling this technique with gas chromatography (GC) and liquid chromatography (LC) detectors makes it an extremely versatile technique.
Within this context, despite the first attempts to couple SFC with electronic ionization mass spectrometry (EI-MS), the use of atmospheric pressure ionization interfaces (API) resulted more feasible, being more compatible with high CO_2 flow.
However, the possibility of obtaining high quality EI spectra for compounds normally not analyzed by GC-MS represents the starting point of the present research work.
A systematic study was carried out for a deep investigation of the influence of each SFC and EI-MS parameter on the performance of the new SFC-EI-MS instrumental setup.
Particularly, the system consists of an SFC coupled to a GC-MS. An UV detector is used during chromatographic method optimization in order to monitor how the processes occurring at the GC-MS interface (actually SFC-EI-MS interface) and into the ion source can affect the separation. Then, the EI-MS detection occurs serially to the UV one *via* a 10 mm fused silica capillary tubing, heated at 200 °C, connected to a Tee Valve which splits the flow rate between the backpressure regulator (BPR) and MS: major is the pressure set at the BPR , major will be the eluent amount entering into the MS. The interface heating contrasts the CO_2 expansion and allows the fast transfer of the analytes into the ion source. Finally, setting the maximum ionization source temperature (300 °C) led to a considerable increase in signal intensity, because of very fast and efficient vaporization/desolvation/ionization phenomena.
The SFC-EI-MS prototype was then employed for a fast, automatic and reliable identification of semi/non-volatile compounds, by the comparison with thousands of spectra present in the commercially available libraries.

P14

A rapid and untargeted characterization of the non-volatile residues of citrus essential oils and the unsaponifiable fractions of vegetable oils was achieved and a spectral similarity major than 90% was obtained for all the compounds.

P15

SIMULTANEOUS DETERMINATION OF EPERISONE HYDROCHLORIDE, PARACETAMOL, AND THEIR METABOLITES IN MOUSE URINE SAMPLES USING HPLC-PDA

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Recently, eperisone hydrochloride was used in association with paracetamol for the treatment of moderate to severe pain. Eperisone hydrochloride is subject to extensive first-pass metabolism, which leads to low concentration in biological fluids. For this reason, sensitive methods are required to evaluate the concentration in different biological matrices.

This work reports a high-performance liquid chromatography-photodiode array (HPLC-PDA) method for the simultaneous analysis of eperisone hydrochloride, paracetamol and its metabolites in mouse urine samples. Samples were collected from mouse housed in metabolic cages after oral drug administered at different incubation times (4, 8, 16 h).

The analyses were carried out using Xtimate C18 column (250 x 4.6 mm, 5 µm) and mobile phase made up from MilliQ water (A) and methanol/acetonitrile mixture (4:3, *v:v*, B) under isocratic conditions (70:30, *v:v*). Mobile phases (A and B) were added with 0.1% (*v:v*) of formic acid. Paracetamol sulfate, paracetamol, paracetamol-d-glucoronate, ethyl-6-fluoro-1-methyl-4-oxo-7-(1-piprazinyl)-4H-[1,3]thiazeto[3,2-a]quinoline-3carboxylate (IS), and eperisone hydrochloride were detected at their maximum wavelengths of 251, 252, 248, 281 and 265 nm, respectively. HPLC-PDA method showed regression coefficient values ≥ 0.9658. All the parameters were calculated according to the International Guidelines for bioanalytical method validation. The optimized retention times of paracetamol-d-glucoronate, paracetamol, paracetamol sulfate, ethyl-6-fluoro-1-methyl-4-oxo-7-(1-piprazinyl)-4H-[1,3]thiazeto[3,2-a]quinoline-3carboxylate (IS), and eperisone hydrochloride were 3.5, 3.9, 4.9, 5.47 and 17.4 minutes, respectively.

The reported HPLC-PDA method is easy, rugged and reproducible for an efficient detection, identification, and quantification of these two analytes and their metabolites and represents efficient tool to evaluate the pharmacological dosages for the reported association.

P16

CHARACTERISATION OF VOCs EMITTED BY MICROPLASTICS DURING DEGRADATION: A COMPARATIVE STUDY BETWEEN DYNAMIC HEADSPACE NEEDLE TRAP DEVICE COUPLED TO GAS-CHROMATOGRAPHY-MASS SPECTROMETRY (DHS-NTD-GC-MS) AND SELECTED ION FLOW TUBE-MASS SPECTROMETRY (SIFT-MS)

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The environmental pollution by plastics debris is raising increasing concerns for the harmful effects that either directly or subtly they exert on living organisms, especially in marine habitats. Most research programs concerning the marine litter issue focused the monitoring of abundance, distribution, size and typology of polluting plastics fragments floating in open sea or deposited in coastal marine sediments.
A recently recognised concerning aspect related to microplastics is the emission of volatile organic compounds (VOCs) from these materials once exposed to the environmental conditions, e.g. heat and UV-light. At present no studies of the VOCs emitted from microplastic are described in literature.
In this study we performed artificial ageing of a set of reference polymers (polystyrene, polypropylene, polyethylene terephthalate, and polyethylene) using a Solar Box system. The materials were selected among the most common polymers representing the majority of microplastics in the environment. VOCs were determined at t_0, after 1, 2, 3 and 4 weeks of artificial ageing using SIFT and DHS-NTD-GC-MS. The aged polymers were placed into two vials (10 mL) and then heated at 60 °C. After 1 hour, the emitted compounds were transferred from one vial into a needle trap device packed with 1 mg of Tenax GR (60/80 mesh) at 5 mL/min for DHS-NTD-GC-MS analyses. Afterwards, NTDs were thermally desorbed at 300 °C in split-mode. The replicate sample in the second vial was used for SIFT-MS analysis. We applied SIFT-MS in full scan mode using the H_3O^+, NO^+ and O_2^+ as reagent ions, with nitrogen as carrier gas.
Selected ion flow tube-mass spectrometry (SIFT-MS) is a direct mass spectrometric technique which achieves real-time, quantitative analysis of VOCs in air at trace levels by applying precisely controlled ultra-soft chemical ionization, and eliminating sample preparation, pre-concentration and chromatography steps SIFT-MS employs different chemical ionization agents generated in situ to react with VOCs in controlled ion-molecule reactions, thus achieving detection limits in the parts-per-billion-by-volume (ppbv) range for most analytes.
In order to evaluate the main modifications induced by degradation we applied a multivariate approach to identify the tendencies of emission of these materials during 4

weeks of artificial ageing. Results highlighted a clear increasing trend over time of VOCs emitted from polymers.
The combination of these methods provides a comprehensive chemical characterization of VOCs emitted by polymers and promises a new possible analytical approach to microplastics analysis.

P17

DEVELOPMENT AND VALIDATION OF TWO ORTHOGONAL HPLC METHODS FOR THE STUDY OF THE ACCELERATED STABILITY ASSESSMENT PROGRAM (ASAP) OF ACETYLSALICYL ACID

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The study concerned two orthogonal HPLC-UV methods developed and validated according to the ICH Q2(R1)[1] guideline applied to the monitoring of the results of an Accelerated Stability Assessment Program (ASAP) protocol performed on acetylsalicylic acid, which was used as model compound.
The ASAP protocols are procedures aimed at evaluating rapidly the stability of a drug substance (DS) or a drug product (DP) in solid state. In these protocols, the effects of heating and exposure to controlled relative humidity percentages (RH%) on the active principle ingredient (API) are studied. The degradation rate of the API under conditions such as 80°C ≥ temperature ≥ 50°C and variable RH% are used to compute a model able to predict by extrapolation the level of API degradation (as DS or DP), in conventional long-term studies. The advantage of the ASAP protocols is to provide preliminary data, usually in less than 1 month, on the outcome of long-term stability studies which normally last 12-24 months[2-4].
By heating acetylsalicylic acid in the presence of water the acetyl ester group undergoes the hydrolysis reaction leading to the formation of salicylic acid and acetic acid and the two developed HPLC methods monitored the degradation reaction progress over time. The first HPLC method in ion-pair reversed phase chromatography used a RP-18 Purospher® column (Hibar® HR, 50 × 2.1 mm, 2 µm, 200 Å) whereas the second one a ZIC®-HILIC column (150 × 2.1 mm, 3.5 µm, 200 Å). The methods allowed the quantification of the degradation products obtained during the ASAP protocol in acetylsalicylic acid samples. Detailed observations regarding the degradation of acetylsalicylic acid modulated by heating and RH% were possible thanks to the use of selective and sensitive HPLC-UV methods. Our experiments on acetylsalicylic acid (DS) samples confirmed that temperature and RH% cause the formation of salicylic acid in consequence of the degradation conditions applied. The degradation rate of the API was greater when it was stored in the presence of humidity levels of 50-75%. In the presence of Drierite® (dehydrating reagent that provides RH <25%), the degradation rate was slower at all temperatures. All the observed differences between the degradation treatments of the API were explained in light of the analytical data acquired. The use of orthogonal validated methods evidenced that the application of the ASAP protocol requires an accurate control of the RH% in the vessels in which the stability tests were performed.

P17

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P18

FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY (FT-ICR-MS) FOR HIGH-RESOLUTION ANALYSIS OF FENNEL PROTEINS

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In recent years, proteomic analyses have played an important role in analytical and food chemistry; in particular mass spectrometry (MS) based methods have been suggested as confirmatory tools for an accurate protein identification in the field of food quality and safety. Emphasis is placed on food processing, in the determination of possible contaminants like bacteria and fungi, and in allergen detection [1,2]. Among the different proteomic strategies, bottom-up analysis remains the workhorse for protein characterization; nevertheless, it results in a greatly increased complexity of the generated peptide mixture, requiring highly sensitive and efficient methods which can lead to correct identifications. A prominent technology for high throughput proteomic analysis is Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry, providing the highest resolving power and mass measurement accuracy. Moreover, the large dynamic range and unmatched sensitivity of FTICR-MS currently provides the highest quality data for protein identification [3,4].
In this study, a rapid and sensitive bottom-up method by FT-ICR is described for the identification of fennel proteins, without prior fractionation. The peptide-level method was previously validated on tryptic digests from ubiquitin standard protein. Ultra-high-resolution mass profiles were acquired using a 12 Tesla FT-ICR mass spectrometer in positive mode via electrospray ionization (ESI). A mass resolving power of around 250,000 was achieved for all spectra while collecting 50 scans per sample with a 4M transient. The method benefits from high resolution which allows to detect proteins in a mass range up to m/z 8000 in a few seconds. The most intense precursor ions were selected for collision-induced fragmentation. The acquired MS and MS/MS datasets were used in the database searching for protein identification. Few microliters of fennel extracts were analyzed after enzymatic digestion with trypsin. Although fennel (*Foeniculum vulgare Mill.*) has attracted attention as a medicinal plant with an enormous amount of health benefits [5], it is recently recognized as an allergenic source, especially in the Mediterranean area. Therefore, this work represents the starting point for allergen characterization in fennel samples, allowing the upgrade of the pattern of allergenic molecules in food products.

P18

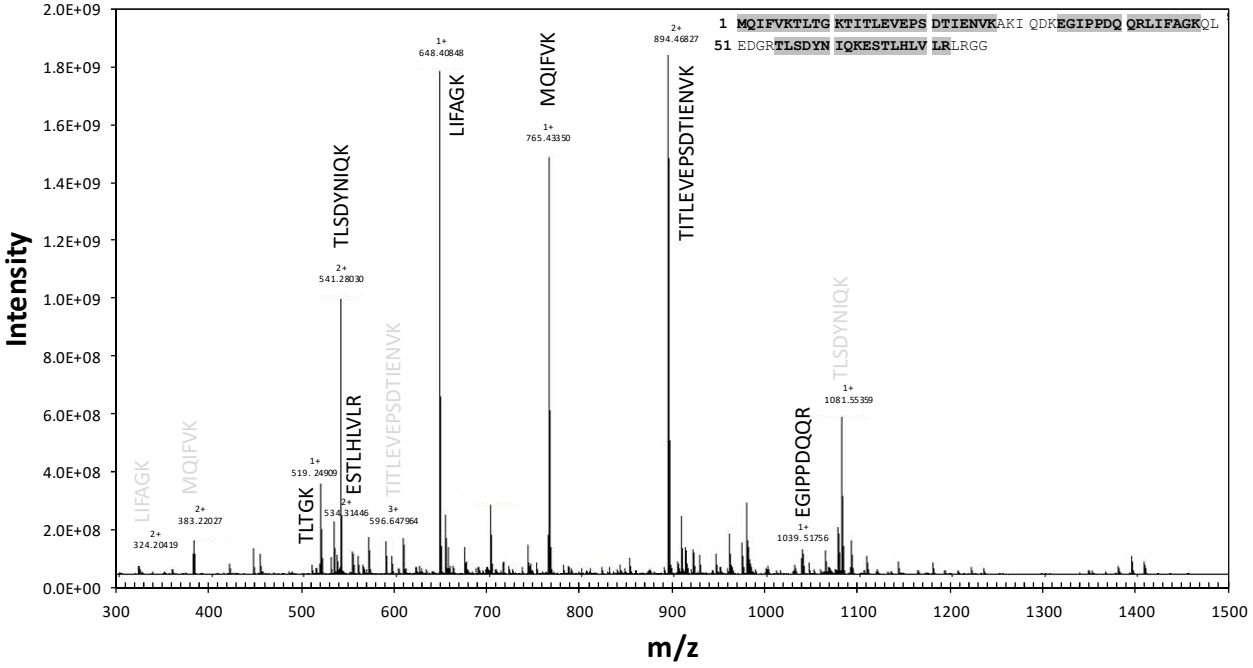

Figure 1. ESI FT-ICR mass spectrum of ubiquitin peptide mixture

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P19

SIMULTANEOUS DETERMINATION OF PSYCHOACTIVE SUBSTANCES IN URBAN WATERS BY MEANS OF dLLME EXTRACTION FOLLOWED BY HPLC-MS/MS ANALYSIS

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The use of illicit drugs represents a social problem worldwide and methods for estimating the extent of drug consumption may be of great interest [1].
Usually information on drug consumption habits is obtained from drug addiction assistance centers, hospital emergency departments, law enforcement investigations or from populational surveys. However, in the last years it was shown that wastewater analysis may represent a suitable tool to explore the drug-taking habits of a population [2]. Wastewater in fact contains a complex mixture of chemical substances that include illicit drugs and products arising from human metabolism [3].
The aim of this work was the development of a multi-class analytical method for the simultaneous determination of twenty drugs of abuse, both including traditional drugs and new psychoactive substances (NPS). The investigated substances belong to different classes with chemical and pharmacological properties such as cannabinoids (natural and synthetic), amphetamines, opiates and metabolites.
The novelty of the proposed approach is the use of dispersive liquid-liquid microextraction (dLLME) for analyte extraction, which allows substantial advantages in term of costs, rapidity and reduction of organic solvent used. dLLME is based on a ternary component solvent system in which an appropriate mixture of extraction solvent and disperser solvent is quickly injected into the aqueous sample with a syringe. In addition, with dLLME very high enrichment factor may be obtained, which is crucial for wastewater analysis.
The analysis was performed by means of LC–MS/MS with a triple quadrupole mass spectrometer in multi reaction monitoring acquisition (MRM) mode, which allowed to obtain optimal performances for the simultaneous analysis of very different analytes. The method was validated according to international guidelines.
Water samples were collected in different sites: three samples were collected in Rome in the Tevere river, while a number of samples were collected in the province of Frosinone, i.e. Sacco and Cosa Rivers. Sample collection was carried out both on Saturday and Sunday in order to estimate the profiles of local drug consumption and possible temporal variations during the weekend. In the collected samples different drugs were identified and quantified, i.e. cocaine, prazepam, amphetamine, benzoylecgonine, diazepam and THC. Concentrations in ng/mL were found suggesting a diffused consumption of these drugs.

P19

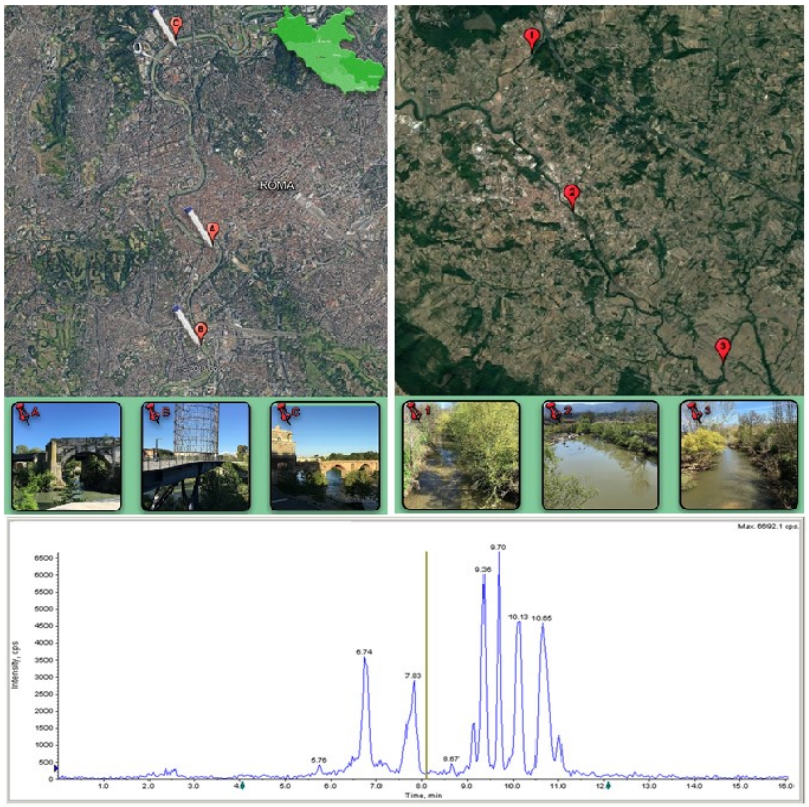

Figure 1. Samples collection sites and example of results (TIC chromatogram).

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P20

AN INTEGRATED MULTIDISCIPLINARY APPROACH TO INVESTIGATE TRACE ELEMENTS SOURCES IN A MOUNTAIN WATERSHED

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Source apportionment of trace elements in waters, with focus on Potentially Toxic Elements (PTEs: i.e., Cd, Ni, Cr), is an issue of major concern regarding environmental chemistry, both in research and decision making [1]. Different approaches can be used to evaluate PTEs sources in waters, but classic single-way approaches are often limited and can easily fail [2].
Therefore, we propose an integrated multidisciplinary approach to understand trace elements sources including: natural background evaluation, seasonal correlations and temporal trends of elements analysis. We then test it on a watershed located in the central Alps.
We collected water samples along the whole melting season (monthly from June to October, in 2014, 2015 and 2016, for a total of 150 samples) and 6 sediment samples. Chemical analyses include major ions (through ionic chromatography) and PTEs quantification (through ICP-MS) for waters, and the quantification of the total load of metals in sediments through acid digestion with *aqua regia*.
The multi-way data processing includes: spatial and temporal trends analysis (focusing on the seasonal trend along the melting season), cluster analysis of variables, and the calculation of a partition index between water and sediment samples to quantify the naturally available PTEs for water dissolution [3].
We then combined the outputs obtained from the different approaches to understand PTEs sources in the analyzed watershed.
Thanks to this approach, we observed the natural occurrence of Fe, Mn, Co, Cr, Ni and As additional atmospheric deposition source for Zn, Cd and Ag has been identified.
Nickel and As, the only elements presenting concerning concentration in water compared to World Health Organization (WHO) standards for human consumption among our samples, interestingly were evaluated as naturally occurring in the study area, highlighting a geochemical anomaly.
Also, redundant observations suggest a possible mixed source on Cu, As and Pb, highlighting the possible erroneous source appointment applying a single-way approach.
This study elucidates the need of an integrated approach to avoid un-necessary or misleading assumptions in the PTEs source appointment. A single-way approach application, in fact, can fail in understanding element source in a complicated and dynamic compartment like surface water.

P20

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P21

SCREENING ANALYTICAL METHOD FOR THE DETERMINATION OF NON DIOXIN-LIKE POLYCHLORINATED BIPHENYLS IN CHICKEN EGGS BY GAS CHROMATOGRAPHY AND ELECTRON CAPTURE DETECTION

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Polychlorinated biphenyls (PCBs) are a group of anthropogenic environmental pollutants with serious implications for human health, due to their great toxicity, bioaccumulative character and resistance to metabolic degradation [1]. Due to their lipophilic nature, PCBs tend to accumulate in fatty tissues, resulting in a widespread distribution along the food chain. Even if the toxicity equivalency factor (TEF, based on the toxicity of the 2,3,7,8-tetraclorodibenzo-p-dioxin as a reference) has been determined only for dioxin-like compounds (DL-PCBs), six non dioxin-like polychlorinated biphenyls (NDL-PCBs, congener 28, 52, 101, 138, 153, and 180) are typically used as indicators to monitor the contamination levels in foodstuffs, due to their higher contents respect to other congeners [2].
Considering the importance of NDL-PCBs determination in food samples, during the last decade efforts have been done to develop accurate methods for determining the levels of NDL-PCBs in food matrices [3,4]. In particular, for high throughput applications in monitoring and risk-assessment studies, screening analytical methods based on electron capture detection [5,6] have been developed to provide quick and reliable results, ensuring at the same time good results in terms of selectivity, instrumental costs and simplicity.
In this work, a sensitive and reproducible screening analytical method for the determination of six non dioxin-like polychlorinated biphenyls (NDL-PCBs, congener 28, 52, 101, 138, 153, 180) in chicken eggs based on accelerated solvent extraction (ASE) procedure for the fat extraction and determination, a solid phase extraction (SPE) sample clean-up process, and a gas chromatography - electron capture detection (GC-ECD) analysis is here proposed. The optimized chromatographic separation, in less than 25 min, returned good responses for the six NDL-PCBs in the range of 2.5–60.0 μg L^{-1}, with correlation coefficients always higher than 0.9995. Instrumental limits of detection were between 0.08-0.35 μg L^{-1}, corresponding to 0.05 and 0.23 ng g^{-1} fat in the matrix, while method detection limits, calculated on spiked egg samples, ranged from 1.6 to 3.5 ng g^{-1} fat. The method has been extensively validated in terms of selectivity, sensitivity, recovery, precision, ruggedness and measurement uncertainty, following the European Directives.

P21

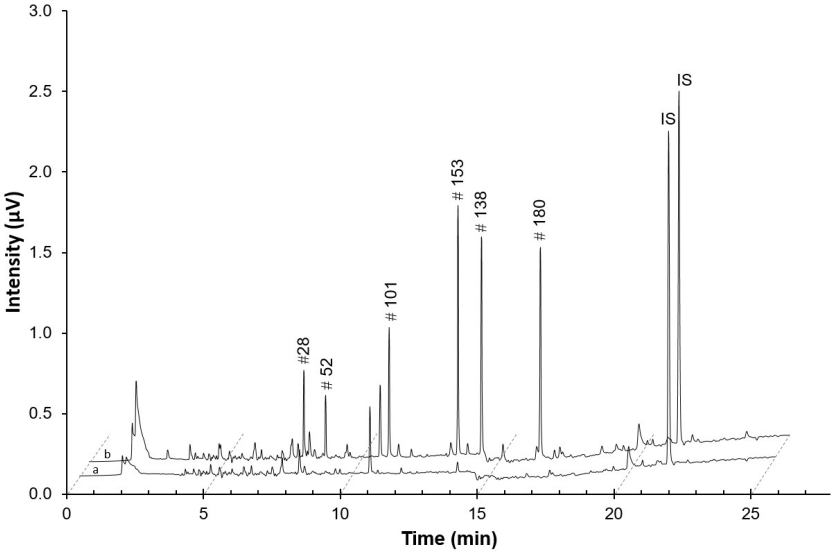

Figure 1. Chromatograms of blank samples (a) and spiked egg yolk sample (b) with NDL-PCBs at 40 ng g^{-1} fat. IS: internal standard (NDL-PCB #209).

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P22

A NEW SPME DEVICE BASED ON A PENCIL-TYPE COATED CARBON FIBERS: FAST ON-FIBER DERIVATIZATION AND GC/MS ANALYSIS OF PHYTOHORMONES IN WHEAT

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Phytohormones, a collection of small signal molecules with various structures, are considered key molecules for the control of a series of physiological processes of plants [1]. Among them, jasmonic acid, indole-3-acetic acid, and abscisic acid have captured a considerable attention of the scientific community for their important role in the plant physiology. In fact, it has been proved that jasmonic acid (JA) regulates plant responses to insect herbivores and abiotic stress [2], while indole-3-acetic acid (IAA) stimulates growth processes such as cell elongation and division [3], and abscisic acid (ABA) controls plant senescence and responses to stress [4].
To evaluate the presence of phytohormones in plants, different sample pre-treatment and/or clean-up methods have been employed: among them, solid-phase microextraction (SPME) [5] has been widely used in the latest years for pretreatment procedures on real samples, due to its simplicity of use, relatively short sample processing times, the variety of available stationary phases, and the possibility to reuse fibers. In this work, new coated carbon fibers (CCFs) have been synthesized, characterized and used as solid phase microextraction (SPME) matrix for the analysis of phytohormones (jasmonic acid, indole-3-acetic acid, and abscisic acid) in wheat samples. The SPME device, realized inserting CCFs in a pencil-type device, when coupled with gas chromatography-mass spectrometry, provides in few steps high recovery values (79 to 112%), fast on-fiber derivatization (30 s), good method reproducibility (RSD < 20%), low detection limits (0.5 – 2.1 ng g^{-1}), and excellent linearity (up to 200 ng g^{-1}). The proposed device can be then considered as a promising and functional tool for fast and reliable extraction and preconcentration of analytes from real samples, allowing a simple derivatization procedure and direct injection in the chromatographic instrumentation.

P22

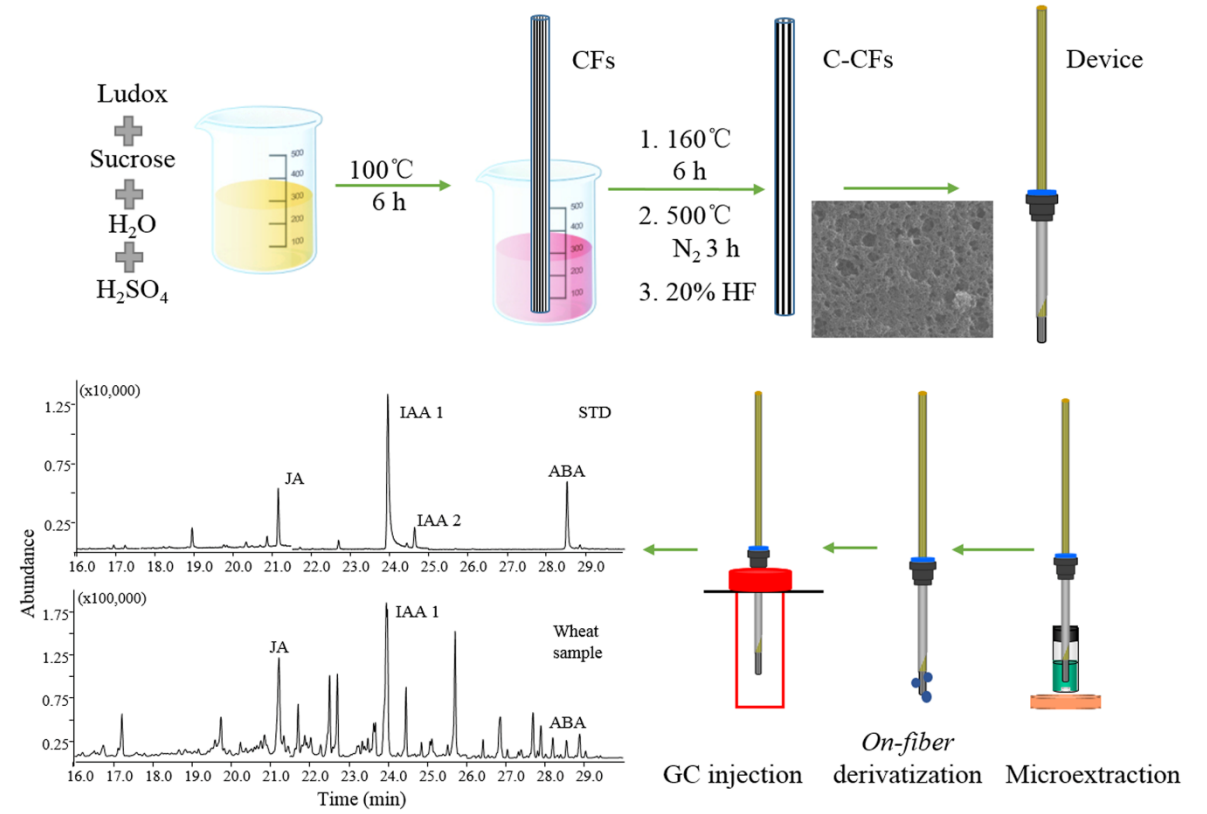

Figure 1. Pencyl-type carbon coated fibers and their use for on-fiber derivatization and GC/MS analysis of phytohormones

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P23

AUTOMATED SAMPLE PREPARATION WORKSTATIONS COUPLED ONLINE TO LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY FOR A COMPREHENSIVE ELUCIDATION OF LIPID PROFILE

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Lipidomics is the metabolomics branch that studies lipids within a living system, aiming to determine specific markers of pathologies or inflammations.
Gas chromatography (GC) methods, which focus on typical ratio between specific components like the n-6:n-3 polyunsaturated fatty acids, are widely accepted within the scientific community.
Recently, the lipidomics approach opened new insight toward the determination of holistic lipid profiles. In fact, monitoring intact lipids can reflect more deeply the dysregulation of lipid metabolism in response to exogenous stimuli and provide elucidations on the perturbation of essential metabolic processes in which each species is involved.
Ultra-high performance liquid chromatography coupled to mass spectrometry (UHPLC-MS) represents the most employed technique in lipidomics. Nevertheless, achieving a fast, exhaustive and reliable identification is still a challenge, due to the not repeatable and poorly informative nature of atmospheric pressure ionization (API) MS techniques, normally hyphenated to LC, that avoid the building and the widespread use of LC-MS databases.
In the present research, a novel linear retention index (LRI) system is proposed as alternative identification method, to be applied as stand-alone tool, as well as in combination with MS data. For such a purpose, the chromatographic resolution acquires primary importance, since the identification will be mostly based on the elution properties. After the building of a database containing more than 200 lipids, different biological and food samples were identified only on the basis of LRIs with a tolerance of ±15 LRI units with respect to the tabulated values, leading to an automatic and correct peak assignment for the majority of analytes.
Finally, the additional strength point is represented by the use of automatic sample preparation procedures, coupled online to LC- and GC-MS platforms, to maximize analytical throughput in both clinical and food application area. Within this context, researcher efforts were addressed to speed up and automatize each step of the entire analytical work-flow.

P24

LINEAR RETENTION INDEX SYSTEM APPLIED TO LIQUID CHROMATOGRAPHY AS NEW APPROACH FOR THE QUALITY CONTROL OF OXYGEN HETEROCYCLIC COMPOUNDS IN ESSENCES, COSMETICS AND FOOD

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In addition to the European Regulation *(EC No 1223/2009)* [1], which sets the maximum amount of psoralens permitted in cosmetics, several agencies have issued their own Opinions in this regard [2,3] on the basis of the last studies about psoralen phototoxicity and the limits imposed by the analytical methods commonly employed for their determination. In particular, the *International Fragrance Association (IFRA)* established high Limits of Quantification in case of PDA detection, suggesting the need of a more selective and sensitive method for the analysis of psoralens in finished cosmetic products [4].
The aim of this research was to demonstrate the validity and applicability of the Linear Retention Index (LRI) approach in liquid chromatography to make the analysis of coumarins (C), furocoumarins (FC) and polymethoxyflavones (PMF), called oxygen heterocyclic compounds (OHC), automatic and reproducible at inter-laboratory level. The LRI system was applied to both HPLC-PDA and HPLC-MS/MS methods as additional parameter with the spectral similarity given by the UV-Vis and MS/MS libraries.
Quantitative data were obtained by external calibration through the acquisition at 315 nm and in Multiple Reaction Monitoring mode, for PDA and QqQ MS detection, respectively.
LOQ lower than those established by IFRA were achieved through the HPLC-PDA method for all target FC. A deep investigation of the obtained results was carried out by the creation of calibration curves in pure solvent and spiking three different distilled essential oils (lemon, bergamot, mandarin) used as blank matrices. The results showed how the LOQ are strongly dependent by the interfering compounds of the matrix, therefore calibration curves on blank matrices represent the best approach for the correct quantitative determination by PDA detector. The HPLC-MS/MS method is useful especially for the analysis of finished cosmetic and food products, where OHC are contained at trace level.
The LRI approach, based on the combination of spectral and retention data, showed its reproducibility in different systems and highlighed its usefulness to guarantee the correct

estimation of FC even at inter-laboratory level and when the method is intended to be fully automatic by using suitable LC software, with integrated LRI function.

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P25

IDENTIFICATION OF EXTRACELLULAR POLYMERIC SUBSTANCES IN EAST ANTARCTIC PACK ICE SAMPLES BY A HPLC-MS/MS METHOD

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Iron (Fe) is the most important trace element in the ocean, as it is necessary for photosynthetic processes related to the phytoplankton growth, playing a role of micronutrient. In particular, Fe plays a crucial role in those areas called High Nutrient Low Chlorophyll (including Antarctica), where the low Fe inputs limit marine productivity. In polar areas, sea ice is fundamental in the biogeochemical cycle of Fe, as it accumulates and stores Fe during winter months and releases it to surface waters after its melting during spring / summer months. This covers a high importance for the biotic component and for the triggering of primary production phenomena in the Southern Ocean.
The organic complexation of Fe in Antarctic pack ice samples was primarily measured by the competitive ligand equilibration adsorptive stripping voltammetry (CLE-AdSV) technique. The results were considered together with other environmental and biological parameters (temperature, salinity, chlorophyll-a, particulate organic carbon (POC) and particulate organic nitrogen (PON) concentration), confirming that sea ice is an environmental matrix of accumulation of dissolved Fe and organic material, especially in the portion closest to sea water (bottom ice).
In this study, a method using High Performance Liquid Chromatography-tandem Mass Spectrometry (HPLC-MS/MS) for the identification and characterization of organic Fe binding ligands in sea ice samples collected in the East Antarctic Region is presented.
The first step was the optimization of the preconcentration procedure of the ligands from the samples by using C18 Solid Phase Extraction cartridge, which allowed us to use a smaller sample volume than other extraction procedures reported in literature.
A Phenomenex Jupiter 5 µm C18 300 Å 150x1 mm column (Torrance, California USA) was used for the chromatographic separation with a mobile phase composed of 0.1% (v/v) formic acid in water and 0.05% (v/v) formic acid in acetonitrile. The HPLC system was interfaced directly to the mass spectrometer by ESI source. All samples were analyzed in positive ionization mode with a scan in the range from 100 to 1500 m/z.
Several Extracellular Polymeric Substances (EPS) have been identified in the samples, which differ in the number of glucose units making up the polysaccharide chain. The presence of these compounds is probably due to the production of mucilaginous substances originated by the phytoplankton and the bacteria associated to sea ice.

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DISCLOSING THE COMPLEX MULTIMATERIALITY OF FELLINI'S STAGE COSTUMES EXPLOITING MASS SPECTROMETRIC TECNIQUES

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Since the second half of 19th century, the great innovation in the industrial field led to the production of many varieties of new synthetic materials, which started to be used for several applications. The world of textiles production was radically changed both from the aesthetic point of view and from the performances and durability of the materials employed. On the one hand, new fabrics, often obtained from modification of natural fibers, were introduced showing the advantages of lower production costs and tailored features. On the other hand, hundreds of synthetic organic dyes and pigments, characterized by bright and appealing hues, were adopted for dyeing the new textiles. This wave of innovation led to industrial products whose chemical proprieties were totally unknown and unexplored.
This work aims at characterizing the composition of two stage costumes used in Federico Fellini (1920-1993) movies. One of the costumes is a tunic worn by a prelate in "Roma" (1927) and the other is the beautiful dress of a lady in "Il Casanova" (1976). The samples were kindly provided by *Opificio delle Pietre Dure* (OPD, Florence), involved in a diagnostic and restoration campaign of several costumes for a special exhibition dedicated to the famous Italian director.
In order to identify the fibers used for the manufacturing of these dresses, a reference database was created analyzing several synthetic fibers by Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) and Pyrolysis Gas Chromatography coupled with Mass Spectrometry (Py-GC/MS). As regards the identification of the dyes, Liquid Chromatography coupled with Diode Array Detector and high-resolution mass spectrometry (HPLC-DAD, LC-ESI-Q-ToF) were employed successfully. The analytical approach adopted allowed us to fully disclose the composition of the costumes constituted by a mixture of synthetic and artificial fibres dyed with complex mixtures of last generation synthetic dyes. Different materials were chosen in accordance to the structure and the aesthetics of the costumes, and various formulations were employed for different hues, possibly obtained by consecutive dyeing baths. The results collected on these multi-material objects were fundamental to fine-tune an effective restoration aimed at removing or minimizing the damages due to ageing or poor conservation conditions. The simultaneous study of both the fibres and the dyes points out the importance of their mutual compatibility and possible interactions especially after ageing. This work highlights as the analysis of several reference materials and case studies is the best approach to characterize the last generation fabrics and dyes, still little investigated up to now.

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ANALYTICAL CHARACTERIZATION OF ANTICANCER PLATINUM (IV) PRODRUGS OXALIPLATIN ANALOGUES

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Some Pt(IV) complexes are octahedral coordination compounds with recognized antitumor properties. They are considered prodrugs, that are activated *in vivo* to Pt(II) complexes with an irreversible reduction mechanism by endogenous intracellular biomolecules (glutathione, ascorbic acid) [1]. The reduction potential of Pt(IV) complexes is influenced by the axial ligands; specifically, it has been found that optimal values for *in vivo* reduction are obtained when axial positions are occupied by carboxylate groups [2]. In this work, six Pt(IV) complexes have been synthesized with the carrier ligand *trans*-1,2-diamine-4-cyclohexene (DACHEX): *cis,trans,cis*-[Pt(OXA)$(OH)_2$(DACHEX)], *cis,trans,cis*-[Pt(OXA)$(AcO)_2$(DACHEX)] (AcO=acetate), *cis,trans,cis*-[Pt(OXA)$(BzO)_2$(DACHEX)] (BzO=benzoate), *cis,trans,cis*-[Pt(OXA)Cl_2(DACHEX)], *cis,trans,cis*-[Pt(OXA)(AcO)(Cl)(DACHEX)], *cis,trans,cis*-[Pt(OXA)(OH)Cl(DACHEX)]. DACHEX possesses a double bond in the diaminocyclohexane ligand present in oxaliplatin, the commercial drug used in the clinics for the treatment of colorectal cancer.
All the complexes were characterized by multinuclear NMR analysis and X-ray photoelectron spectroscopy (XPS) to determine Pt chemical oxidation state. Moreover, the complexes were electrochemically characterized by cyclic voltammetry to evaluate the possibility of reduction *in vivo*. Reduction potentials were found in the range -1.25 – 0.08 V, these values being compatible with *in vivo* reduction and consistent with those reported in the literature for analogous Pt(IV) complexes [2]. Perspective studies will be focused on the evaluation of *in vitro* cytotoxicity against human cancer cell lines.

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FPSE-HPLC-PDA ANALYSIS OF SEVEN PARABEN RESIDUES IN HUMAN WHOLE BLOOD, PLASMA, AND URINE

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Parabens represent alkyl esters of *p*-hydroxybenzoic acid (PHBA), widely used as preservatives and antimicrobial agents in food, pharmaceutical products and cosmetic products. These compounds are easily absorbed in the human body, so that recent studies have classified parabens as endocrine disrupting compounds (EDCs), which is products that interfere with hormone production, release, transport, metabolism or elimination [1]. The use of parabens is currently limited by numerous institutions for which interest is growing in the development of new and increasingly sensitive methods for their determination in environmental, pharmaceutical, cosmetic and health products

This work describes an innovative, fast and sensitive method for the simultaneous determination of seven paraben residues including methyl paraben (MPB), ethyl paraben (EPB), propyl paraben (PPB), isopropyl paraben (iPPB), butyl paraben (BPB), isobutyl paraben (iBPB) and benzyl paraben (BzPB) in human whole blood, plasma and urine. The analytes were extracted from the biological matrix by an innovative technique, fabric phase sorptive extraction (FPSE) and subsequently analyzed by high-performance liquid chromatography (HPLC) coupled with photo diode array (PDA) detector.

The separation was conducted using a Spherisorb C18 column with methanol and phosphate buffer as mobile phases. The analytical method has been validated according to the International Guidelines through the construction of a calibration curve for each biological matrix and considering precision (intra and inter day), accuracy, selectivity, LOD, LOQ and robustness. Subsequently, the performance of the analytical method was evaluated on real biological samples collected from healthy volunteers.

The proposed method allows the simultaneous analysis of seven paraben residues in three different complex matrices, including whole blood, and therefore it is easily applicable to monitor these substances in different biological sample matrices; furthermore, extraction technique used in this work is fast, easy and in accordance with the new green analytical chemistry (GAC) principles.

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COMPARISON BETWEEN EXHAUSTIVE AND EQUILIBRIUM EXTRACTION USING DIFFERENT SPE SORBENTS AND SOL-GEL CARBOWAX 20M COATED FPSE MEDIA

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This paper reports the performance comparison between the exhaustive and equilibrium extraction using classical Avantor C18 solid phase extraction (SPE) sorbent, hydrophilic-lipophilic balance (HLB) SPE sorbent, Sep-Pak C18 SPE sorbent, novel sol-gel Carbowax 20M (sol-gel CW 20M) SPE sorbent, and sol-gel CW 20M coated fabric phase sorptive extraction (FPSE) media for the simultaneous extraction and analysis of three inflammatory bowel disease (IBD) drugs that possess logP values (polarity) ranging from 1.66 for cortisone, 2.30 for ciprofloxacin, and 2.92 for sulfasalazine. Both the commercial SPE phases and *in-house* synthesized sol-gel CW 20M SPE phases were loaded in SPE cartridges and the extractions were carried out under an exhaustive extraction mode. FPSE was carried out under an equilibrium extraction mode. The drug compounds were resolved using a Luna C18 column (250 mm × 4.6 mm; 5 µm particle size) in gradient elution mode within 20 min and the method was validated in compliance with International Guidelines for the bioanalytical method validation. Novel *in-house* synthesized and loaded sol-gel CW 20M SPE sorbent cartridges were characterized in terms of their extraction capability, breakthrough volume, retention volume, hold-up volume, number of the theoretical plate, and the retention factor. Among the different sorbent phases tested, commercially available phases showed superior results at higher concentration levels for the tested compounds, whereas the new sol-gel CW 20M at low concentrations exhibited interesting results.

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USE OF A NOVEL CONSUMABLE-FREE THERMAL MODULATOR WITHIN THE CONTEXT OF COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY: GAS FLOW OPTIMIZATION ASPECTS

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The present research is based on the evaluation of a novel comprehensive two-dimensional gas chromatography (GC×GC) consumable-free thermal modulator, which is defined as solid-state modulator (SSM). The transfer device is a moving modulator, installed on top of the GC oven and does not require heating and cooling gases to generate GC×GC data. The SSM is formed of a thermoelectric cooling (TEC) device located between two heated aluminum chambers (hot entry and hot exit). The two chambers are each linked to two transfer lines, enabling connections to the GC oven and to the TEC device. The accumulation and re-mobilization steps occur on a trapping capillary, this being subjected to thermoelectric cooling in the middle of the device and micathermic heating in the two adjacent hot zones. The modulation column moves according to a pre-set modulation period to achieve dual-stage thermal modulation.
The SSM is a novel form of modulation and as a consequence, details on optimization aspects are lacking. The present study can be located within such a context. Specifically, the modulation performance was evaluated in relationship to average gas linear velocity values by using different coated trapping capillaries. Detailed information will be discussed on gas flow optimization, with emphasis directed to the efficiency of band re-injection onto the second dimension column.

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PRECISION MEDICINE: ^{1}H-NMR CHARACTERIZATION OF URINARY DIRECT ACTING ANTIVIRAL RIBAVIRIN METABOLITES IN HCV PATIENTS WITH SEVERE LIVER DISEASE

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Chronic hepatitis C (CHC) represents a public health problem, affecting more than 71 million of individuals worldwide. Hepatitis C virus (HCV) infection causes liver inflammation and progressive liver fibrosis, leading to cirrhosis and its complications, such as hepatocellular carcinoma (HCC) [1]. Recently, direct-acting antivirals (DAAs) that act as inhibitors of N5SA, or polymerase, or protease have been shown to result in shorter duration of therapy, better efficacy and tolerance than the interferon whose antiviral action consisted in a progressive immune-mediated elimination of infected hepatocytes. Ribavirin (RBV), the common name of 1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide, is a pro-drug commonly used in DAA treatment of HCV-patients. It's a structural analog of nucleosides and it is phosphorylated intra cells to its active and polar metabolites which are either retained in the cells or dephosphorylated back to ribavirin [2]. Despite the efficacy experienced in difficult-to-cure HCV patients, the administration of RBV was limited since it could cause a toxicity-induced anemia in treated patients [3]. For this reason, the appropriate individual administration of RBV is essential to manage its several adverse reactions and clinical toxicity.

The aims of this work were to identify and quantify pro-active and inactive urine metabolites of RBV by ^{1}H-NMR and to characterize the individual profiles of HCV patients receiving DAAs, depending on high and low inactivation metabolic ability. Urine samples from thirty-one HCV patients with severe liver disease (19 males, aging 47-76 years) were analyzed by high-resolution proton nuclear magnetic resonance spectroscopy and the structural assignment RBV metabolites was carried out by ^{1}H 1D, homonuclear and heteronuclear 2D NMR spectra. Patients were examined at the baseline and after four weeks of DAA treatment. On the basis of TOCSY, HSQC and Pearson correlations, it was possible to identify the structures and distinguish RBV signals from its active (T-$CONH_2$, TR-COOH) and inactive (T-COOH) metabolites. Furthermore, the quantitative analysis of ribavirin and its active metabolites was carried out and the internal ratios of the metabolites and RBV were then calculated. T-$CONH_2$ and TR-COOH were detected in 84.2% (16 out of 19) and in 89.5% (17 out of 19) of

patients, respectively, the unaltered pro-drug RBV in 89.5% of patients (17 out of 19), while the T-COOH metabolite was identified in all the examined samples, although its quantification was not possible due to its low concentration.
The results obtained showed a high variability of the RBV metabolism in HCV patients, in particular concerning the levels of the inactive form. In conclusion, we suggest that the different individual metabolic activities leading to drug pro-active and inactive forms should be strictly linked to therapy effectiveness, leading to the adjustment of the dose toward a personalized regime.
These personalized treatments may result in reduced side effects. Long-term and real-time diagnostic monitoring of results will further contribute to a personalized health care proposal.

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RAPID AND CONVINIENT MEASUREMENT OF CLINICALLY-IMPORTANT AMINO ACIDS IN BIOLOGICAL FLUIDS

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Precision medicine – the ability to precisely tailor treatment to each specific patient – would be significantly advanced by the availability of technology supporting the rapid and convenient, measurement of drugs and biomarkers at the point-of-care. Due to their low cost, ease of use, and good analytical performance in complex clinical samples Electrochemical aptamer-based (E-AB) sensors appear a promising means to this end. Thus motivated, we present here the development of E-AB sensors for the measurement of the aromatic amino acids phenylalanine and tryptophan [1], which are diagnostic biomarkers indicative of a number of metabolic and mental health disorders [2]. The sensors employ previously reported DNA aptamers able to recognize the complexes formed between the amino acid and a rhodium-based amino-acid receptor [3]. We adopted these into the EAB platform by truncating them (causing them to undergo a binding-induced conformational change), modifying them with a redox-reporting methylene blue, and covalently attaching them to an interrogating electrode. We then adapted the system to a "dual-frequency" approach (recently published by our group [4]) that supports *calibration-free* measurements. The resulting sensors are, with only a single dilution step, able to measure phenylalanine and tryptophan in minutes in biological fluids such as blood or urine without significant interference from other amino acids. The speed and convenience with which this is achieved suggests that the E-AB platform could significantly improve the ease and frequency with which metabolic diseases are monitored.

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SEQUENTIAL MULTI-BLOCK METHODS FOR SOLVING CLASSIFICATION PROBLEMS IN FOOD ANALYSIS

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Due to the development of novel technologies and the relatively higher availability of instrumentations, it has become more and more frequent to handle multi-block data sets. For instance, this can happen when the same objects are investigated by different analytical techniques or at different time points. Examining this kind of data, it is more efficient to analyze all the matrices together by means of multi-block (or data fusion) methodologies, rather than to apply individual approaches for each set of measurements.
In the last years, several data fusion strategies have been proposed into the literature, each of them introducing different ways of solving the main limitations that affect the classical methods. A family of multi-block approaches, the sequential methods, have been widely exploited because of some special benefits they provide. In this context, two (relatively) new methods have been developed: Sequential and Orthogonalized Partial Least Squares (SO-PLS) [1-2] and Sequential and Orthogonalized Covariance Selection (SO-CovSel) [3]. Both of them, conceived as regression methods, have been extended to the classification field by combination with the Linear Discriminant Analysis (LDA). Even though these approaches can be used to analyze data of any kind, they have been widely exploited in food analysis.
Among the different applications in this context, one example over the traceability of red garlic will be discussed in detail. Several factors may influence the characteristics of this agro-food, one of the most relevant among them being the growing area; as a consequence, checking the origin of this product is a relevant task. In the light of this considerations, it has been investigated whether it would be possible to trace red garlic by Mid Infrared Spectroscopy (MIR) coupled with multi-block chemometric classifiers. Red garlic samples harvested in four different geographical areas (Castelliri, Proceno, Nubia and Sulmona) were analysed by MIR spectroscopy: per each clove, spectra were collected before and after the removal of the tunic. Eventually, classification models were built in order to assign samples to the four different geographical origins. In order to achieve this goal, the two data blocks (i.e., the set of signals collected on the tunics and the spectra derived by the analysis of un-skinned cloves) were individually handled by a classifier called Partial Least Squares Discriminant Analysis (PLS-DA) and by SO-CovSel-LDA and SO-PLS-LDA.
Finally, the best results, in terms of predictions (on an external validation set of samples) were provided by SO-PLS-LDA. In fact, this approach allowed correctly assigning 19 over 20 test samples (leading to a total classification rate of 95%).

In Figure 1 samples are projected onto the space of the first two SO-PLS-LDA latent variables. From this representation is clearly possible to recognize the four groups of samples. In fact, LV1 allow distinguishing samples belonging to Class Castelliri (red circles, at negative values) from objects belonging to Class Proceno, Sulmona and Nubia (blue squares, green triangles and black diamonds, respectively) while samples appertaining to Class Proceno are distinguished from those belonging to Class Sulmona along LV2.

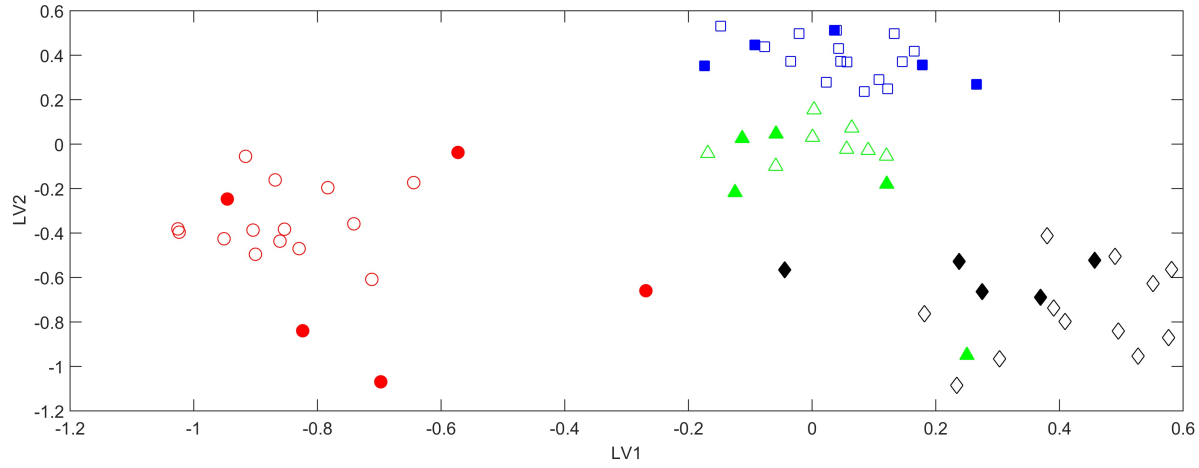

Figure 1. SO-PLS-LDA analysis: Samples project onto the first to canonical variates. Legend: Red circles: Class Castelliri; Blue squares: Class Proceno; Black diamonds: Class Nubia; Green triangles: Class Sulmona. Empty and filled symbols represent training and test samples, respectively

As confirmed by the above-mentioned example, multi-block approaches usually provide better results than the individual analysis of the data blocks. Consequently, handling multi-block sets of data, it is highly recommended to jointly model all the available blocks applying specific methodologies conceived for this kind of data sets, rather than using classical methods on the individual measures.

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PRESENCE OF HEAVY METALS IN COCOA AND CHOCOLATE AND THEIR CHARACTERIZATION

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Cocoa, necessary to produce chocolate, coming from South America, Central America, Africa or Asia, can have similar general composition, but often differences can be found depending on its origin. In this paper commercial samples of cocoa and bitter chocolate produced from different countries are analyzed.
The aim of this work is the comparison between the two products and the determination of eventual presence of potentially toxic cations. For this purpose different parameters are investigated as protein nitrogen, ashes, fatty acids and analysis of ashes.
The correlation between nitrogen protein, ashes and fatty acids shows interesting evidences relative to countries geographically on the same parallel, even if of different continents [1].
To determine concentrations of heavy metals, ashes dissolved in HCl are analyzed with different procedures, according to the concentration range of the following cations: copper (II), lead (II), cadmium (II), zinc (II), arsenic (III), selenium (II), mercury (II).
The analysis is carried out by means of Atomic Absorption Spectrophotometry (AAS), but applying different techniques. AAS in flame (air acetylene) allows obtaining evaluable results for copper (II) and zinc (II), while it was necessary to use graphite furnace for lead (II) and cadmium (II) because they have a lower concentration. The determinations of arsenic (III), selenium (II) and mercury (II) show particular difficulties and it was necessary to use a specific devise involving the so-called hydrides kit.
By comparing the obtained results with the limits settled by both the Italian and the European legislation, it could be deduced that several of the examined samples suffered of heavy toxic metals pollution. From an inspection of the obtained results, it seems possible to find a dependence of pollution on the country producing cacao. It can be supposed that the found cations are absorbed from the respective soil.
The results of analysis will be compared with the limits provided by law. However, the presence of trace component in chocolate could be attributed to a violation of the rules relative to fabrication norms and commerce of cocoa and chocolate already known [2].

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DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR THE SIMULTANEOUS DETERMINATION OF TEN N-NITROSAMINES IN MEAT PRODUCTS BY ANION EXCHANGE POLYMERIC SORBENT EXTRACTION AND HPLC/UV-DAD

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The potential carcinogenicity effects of red and processed meats are at the heart of the scientific community debate for many years [1]. In 2015, the International Agency for research on Cancer (IARC) of the World Health Organization evaluated the carcinogenicity of both red and processed meat. Processed meats were classified as carcinogenic to humans (Group 1) [2]. Among mechanisms and molecules involved in the carcinogenicity induced by consumption of processed meats, the N-nitrosamines (N-NAS) were indicated among the most significant. Indeed, these compounds derive under acidic conditions (i.e. human stomach) from nitrites (added in these products as food preservatives) and secondary amines.

A reference method standardized for the determination of N-NAS in meat products has not been established yet. This type of analytical determination maybe accomplished by using gas or liquid chromatography coupled to mass spectrometry. However, other than expensive, the use of mass spectrometry may be complex, especially for small laboratories and/or quality control laboratories of meat companies.

In this work, a novel analytical method based on HPLC coupled to UV diode array detection for the determination of ten N-nitrosamines (N-nitrosopiperidine, N-nitrosomorpholine, N-nitrosodi-n-butylamine, N-nitrosodiethylamine, N-nitrosodiphenylamine, N-nitrosodi-n-propylamine, N-nitrosopyrrolidine, N-nitrosomethylaniline, N-nitrosomethylethylamine and nitrosodibenzylamine) in meat products was developed and validated.

The method development consisted in the chromatographic separation of ten N-nitrosamines and in the optimization of an effective procedure of sample extraction/purification. The complete chromatographic separation of ten N-nitrosamines, characterized by good peaks symmetry and resolution (Figure 1A), was accomplished by using C_{16} RP column (Acclaim^{TM} PolarAdvantage, 5µm, 250 × 4.6mm, 120Å), eluted by gradient of water and acetonitrile. The absorbance signal was detected at 230 nm.

Different extraction procedures and SPE protocols were compared. The extraction of N-nitrosamines from samples (chicken, beef and swine fresh meats) was optimized comparing the recovery percentages obtained analyzing spiked samples.

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The most effective procedure of sample extraction/purification was achieved using 10^{-2} M HCl as solvent and ultrasonic bath/magnetic stirrer double extraction. Suitable purification of samples was obtained using anionic exchange Strata^{TM} X-AW columns (Figure 1B).
The method validation, performed by an *in-house* model according to the Decision 2002/657/EC and Regulation 2017/625/EC, provided good results (table 1), with mean recovery percentages in the range 80.8%-95.1% and mean CVs lower than 14.4%, demonstrating the suitability of the proposed method in determining ten N-nitrosamines in meats [3].

Nitrosamine	Determination coefficient (r^2)	LOD (µg kg^{-1} in matrix)	LOQ (µg kg^{-1} in matrix)	Mean recovery%*	Mean CV%*	Expanded measurement uncertainty %**
N-Nitrosomorpholine	0.996	80.8	266.7	80.8	14.4	12.2
N-Nitrosomethylethylamine	0.993	105.7	348.9	92.5	4.3	14.6
N-Nitrosopyrrolidine	0.999	20.1	66.2	88.2	11.2	13.4
N-Nitrosodiethylamine	0.997	65.9	217.5	91.7	5.0	8.8
N-Nitrosopiperidine	0.996	77.0	254.1	95.1	6.8	7.8
N-Nitrosodipropylamine	0.999	41.6	137.2	84.8	8.4	9.0
N-Nitrosomethylaniline	0.999	25.8	85.2	93.2	5.4	7.0
N-Nitrosodibutylamine	0.999	35.8	118.1	89.8	4.4	6.8
N-Nitrosodiphenylamine	0.999	46.5	153.4	93.8	4.8	9.8
N-Nitrosodibenzylamine	0.992	111.6	368.3	94.9	7.3	9.6

* Evaluated at three fortification levels: 0.5, 1.0, 1.5 mg kg^{-1} (n=6 for each level)
** Covering factor k = 2

Table 1. Validation parameters of optimized method.

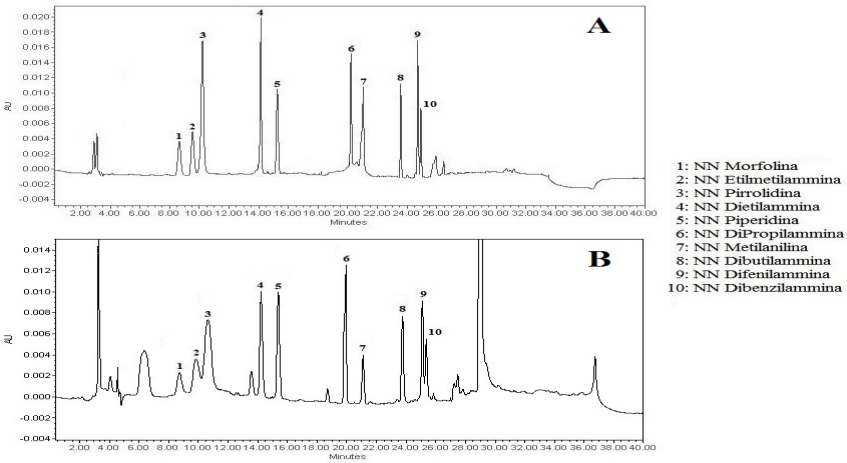

Figure 1. Chromatograms of 10 N-Nitrosamines standard solution (2.0 mg L^{-1}) (A) and chicken fresh meat spiked with 10 N-Nitrosamines at 1.5 mg kg^{-1} (B).

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COMPARISON BETWEEN MONIER-WILLIAMS REFERENCE METHOD AND DIRECT ION CHROMATOGRAPHY FOR DETERMINING SULPHITING AGENTS IN RAW AND PROCESSED MEAT

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Sulphiting agents (SAs) are a class of compounds used as food preservatives and antioxidants. Due to toxic and pseudo-allergenic effects that these compounds can have in humans, the legislation restricts their use. In fresh meat preparations (fresh sausages, hamburger, minced meats, etc.) the SAs are not admitted. However, many cases of "sulphiting treatment" of fresh meats were verified during official controls both in Italy and other countries [1, 2]. The Monier-Williams (M-W) method, optimized for the analysis of meat, is the most employed analytical procedure for the determination of SAs. This procedure was called into question by several authors due to some interfering compounds present in meat that can be converted into sulphate, the ion determined for the indirect quantification of sulphite by the M-W method. An alternative method allows the direct determination of sulphites by ion chromatography with conductometric detection (DIC). This method was fully validated and accredited at Chemistry Department of Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata [3].
In this work, the M-W method was modified, improved, validated and then compared to the method by ion chromatography/conductometric detection for the determination of SAs in raw and processed meats. The improvement, already proposed by different authors but never validated, consisted in replacing the final titration by ion chromatography determination of sulphate ion. The comparison was based on the most important validation parameters (detection limits, linearity, selectivity, repeatability and precision) other than measurement uncertainty and time/cost for a single analysis. The improved M-W method was characterized by sensitivity suitable for this type of determination (LOQ < 10.0 mg kg^{-1} of SO_2) and good reproducibility, not always guaranteed by manual titration. Regarding methods comparison, linearity, repeatability/precision at 40 and 80 mg kg^{-1} of SO_2 and measurement uncertainty were comparable. The LOD and LOQ of M-W method were slightly higher than DIC method. Repeatability and precision at the lowest fortification level (10 mg kg^{-1} of SO_2) resulted higher for the M-W method, but this parameter maybe influenced by traces of some sulphur-containing compounds that may be present in meats. Concerning method selectivity, spiking tests with some sulphur-containing compounds demonstrated that sulphide, 2-methyl-3-furanthiol and L-methionine may cause "false-positive" responses (SO_2 concentrations higher than the legal limit of 10.0 mg kg^{-1}) by using M-W based methods

(Figure 1) (Table 1). This result represents a limitation in the use of M-W based methods as confirmatory methods for the determination of sulphites in raw and processed meats. This limitation is avoided by using the DIC method [4].

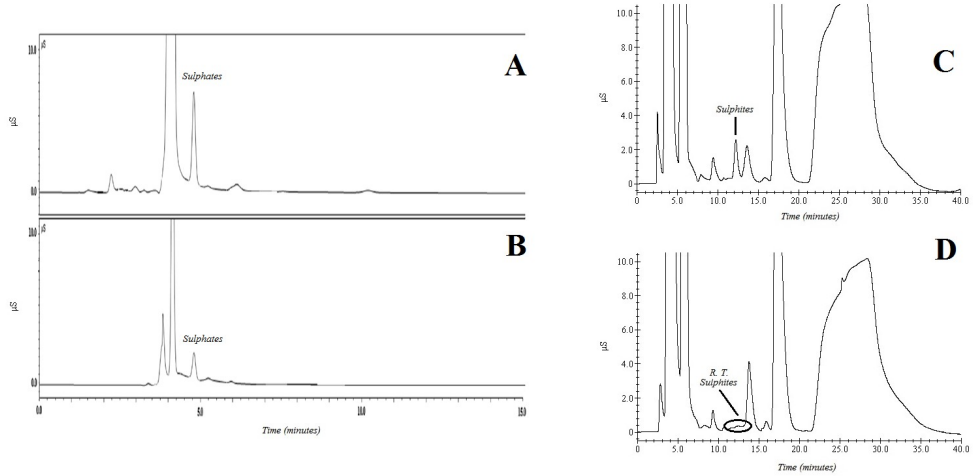

Figure 1. Chromatograms comparison. Cattle fresh raw meat samples spiked with sulphites (80 mg kg^{-1} as SO_2): M-W method (A), DIC method (C). Cattle fresh raw meat samples spiked with sulphides (30 mg kg^{-1}): M-W method (B), DIC method (D).

Validation Parameter		M-W	DIC
Linearity (r)		0.9996	0.9998
Limit of Detection (LOD) (mg kg^{-1} of SO_2 in matrix)		3.3	2.7
Limit of Quantification (LOQ) (mg kg^{-1} of SO_2 in matrix)		9.9	8.2
Measurement range (mg kg^{-1} of SO_2 in matrix)		9.9 - 160	8.2 - 160
Specificity		Not confirmed for sulphide, L-methionine and 2-methyl-3-furanthiol	Confirmed
Precision (as CV%, n=6)	***10 mg kg^{-1} (as SO_2)***	2.4	6.0
	40 mg kg^{-1} (as SO_2)	6.0	6.0
	80 mg kg^{-1} (as SO_2)	5.4	5.3
	Mean precision	4.6	5.8
Recovery percentage (n=6)	***10 mg kg^{-1} (as SO_2)***	93.2	92.1
	40 mg kg^{-1} (as SO_2)	77.1	88.4
	80 mg kg^{-1} (as SO_2)	72.7	85.2
	Mean recovery	81.0	88.6
Measurement Uncertainty (relative percentage)		9.4	9.9
Sample preparation time for a single analysis (minutes)		80	45

Table 1. Comparison of validation parameters obtained for the two analytical methods

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DIFFERENT ANALYTICAL METHODS APPLIED FOR ANALYSIS OF "SANGIOVESE" RED WINE

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Polycyclic aromatic hydrocarbons (PAHs) represents contaminants widely present in foods and beverages. PAHs contaminate soul and air and could alter the organoleptic properties and compositions of farm and commercial products [1]. Grapes, used to produce red wine, can be potentially exposed to PAHs and these contaminates can alter its taste and fragrance. Analytical chemistry can represent a useful tool to monitor and quantify the presence of PAHs in foods and beverages, particularly in red wine [2]. In this contest, different analytical methods, i.e. high-performance liquid chromatography, heavy metal detections, as well as others, were used as "fingerprint" to detect and identify these contaminants.
The aim of this work is the qualitative and quantitative analysis of PAHs, heavy metals, metabolites present in the red wine by different analytical methods. Colorimetric properties of the related grapes have been also investigated. "Sangiovese" red wine was used for the analysis and collected from different Italian regions, winemakers and year of productions. Aliquots of red wine (40 mL) were extracted before the analysis and the extraction procedure was optimized using central composite design (CCD) according to the international standard guidelines. CCD allows to increase the identification of PAHs that potentially contaminate the commercial Sangiovese red wines, as well as improving its recovery. The limits of quantification (LOQ) of the method were 10 µg/mL (acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene) and 25 µg/mL (benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(j)fluoranthene, benzo(a)pyrene, and 3-methylcolanthrene), respectively. The matrix matched standard curves of the various analytes showed good linearity up to 400 µg/mL, with correlation coefficients ≥ 0.9521 for all of them. Intra-day and inter-day precision (R.S.D.%) values were ≤14.7% and ≤14.5%, while the intra-day and inter-day trueness (bias

%) values were in the range from –14.4% to 3.74%. Voltammetric curves of heavy metal contaminants have been recorded by using an Amel Model 433 multipolarograph, equipped with a conventional three-electrode cell. Hanging mercury drop electrode (HMDE) was used to detect Cu(II), Pb(II), Sn(II), Sb(III), Cd(II), Zn(II), while gold electrode (GE) [3] to detect Hg(II). An Ag|AgCl|Cl-satd electrode and platinum wire were selected as reference and auxiliary electrode, respectively. The reported method allowed to quantify PAHs, heavy metals, metabolites, and monitor the colorimetric properties of grapes in commercial "Sangiovese" red wines.

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P38

EVALUATION OF ANALYTICAL PERFORMANCES OF QUARTZ CUVETTES AND DISPOSABLE GLASS VIALS FOR THE DETERMINATION OF FAME AND TAGs IN EXTRA VIRGIN OLIVE OIL

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The main goal of the present study was to evaluate the analytical performances of quartz cuvettes and disposable glass vials while collecting Near InfraRed spectroscopy (NIR) spectra of extra virgin olive oils (EVOO), in order to develop Partial Least Squares (PLS) regression models for the prediction of fatty acid methyl esters (FAMEs) and triacylglycerols (TAGs).
In the last years, the use of spectroscopic techniques, such as NIR combined with chemometric methods has been recognized in various analytical applications for the study of extra virgin olive oil composition. Quartz cuvettes are usually used for NIR spectra acquisitions, resulting in time-consuming measurements, especially in the washing phase, requiring a significant consumption of solvents to remove residuals. For these reasons, it looks interesting to compare the analytical performances of quartz cuvettes and disposable glass vials, which would allow to greatly reduce acquisition times; nevertheless, their analytical performances in EVOO analysis have not yet been investigated. In order to reach this goal, the predictive abilities of PLS regression models for evaluating olive oil quality parameters – such as fatty acid methyl esters (FAMEs) and triacylglycerols (TAGs) – were compared. Samples were provided by AGER foundation under the VIOLIN project (Project code: 2016-0169). 74 PDO extra virgin Italian olive oils have been analyzed by means of NIR spectroscopy using both quartz cells with 5 mm path length and mono-use glass vials provided by BUCHI (Buchi Italia s.r.l.,Milan, Italy). NIR spectra were acquired in the transmission mode with a FT-NIR spectrophotometer (Buchi NIRFlex N-500), in the 4000-10000 cm^{-1} range at a 4 cm^{-1} resolution. All the measurements were performed at a controlled temperature (35°C). Samples were acquired in duplicate and the average spectra were used for data analysis.
For all NIR spectra, before building the calibration model using the PLS1 regression method, two row data pre-treatments, the Standard Normal Variate (SNV) transform [1] and Orthogonal Signal Correction (OSC) [2], were applied. As a first step, Principal Component Analysis (PCA) was performed as a multivariate display method to visualize the data structure and to remove outliers, then PLS was applied in order to predict the amount of FAMEs and TAGs in EVOO samples. For each PLS model, the variance explained by the model, the error in cross validation (RMSECV) and the Ratio Performance to Deviation (RPD) were evaluated. As an example, the predicted versus the measured responses for one FAME

(C16:00) obtained with both the quartz cuvettes and the mono-use glass vials are shown Figure 1.

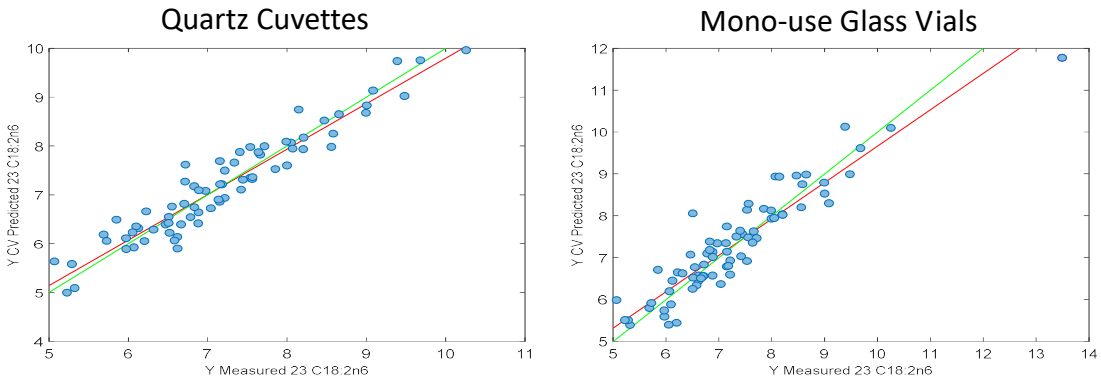

Figure 1. Predicted vs. measured response for (C16:00) with quartz and mono-use glass vials

To verify if the differences among the two types of cuvettes are statistically significant, the Passing-Bablok regression method was applied performing a joint test on slopes and intercepts. Generally, NIR spectroscopy coupled with regression models was confirmed to be a valuable tool for the evaluation of quality parameters of EVOO. In terms of prediction accuracy of PLS models, better performances were obtained from quartz cuvettes, though, Passing-Bablok test suggesting comparable results for both of the types of cuvettes.

This work has been supported by AGER 2 Project, grant n° 2016-0169

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ANALYTICAL METHODS FOR THE ANALYSES OF FOOD LIPIDS BASED ON HIGH RESOLUTION CHROMATOGRAPHY TECHNIQUES

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This paper presents various analytical methods, based on high resolution chromatographic techniques, applied to the study of the composition of different food matrices with a predominantly triglyceride composition (butter, oil, lard). Furthermore, the minor lipid component contained in other types of food, such as flour and semolina is also analyzed. In these food lipids wax and steryl esters were analysed. Moreover, a method based on gas-chromatographic analysis is presented for the quantification of cholesterol content in eggs. For the determination of the fatty acid composition in food matrices with predominantly triglyceride composition, two innovative methods of transesterification of triglycerides in pentyl esters and phenethyl esters of fatty acids are presented. Pentyl esters avoid the loss of butyric acid when it is derivatized as methyl ester in GC analysis and phenethyl esters allowed to separate unsaturated fatty acids without degradation if compared to GC analysis.

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P40

TOTAL PHENOLIC COMPOUNDS IN SEEDS OF SOME APULIAN LEGUME LANDRACES

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Apulia, the most south-eastern region of Italy, has always been an agricultural region. In this region as elsewhere, legumes have had a noteworthy role being a cheap source of vegetable proteins for local people. Moreover, they have been systematically cultivated in succession with cereal and forage species, being able to replace the nitrogen that wheat takes away from soil. Over the time Apulian farmers selected a lot of landraces well adapted to the microclimatic conditions of the different regional landscape environments (i.e.: Monti Dauni, Murgia, Valle d'Itria, Salento, etc.). Some of these landraces are still under cultivation in the traditional environments.

In addition to a valuable protein content, legume seeds contain several bioactive compounds which may have a positive role against important diseases. Phenolic compounds are one of bioactive class of molecules present in legume seeds. Various studies have proved that many phenolic compounds have several biological properties such as antioxidant and anti-inflammatory activities [1-2]. Still today, the content of total phenolic compounds (TPCs) in the Apulian legumes landraces is under investigated. To acquire preliminary information on this topic, the level of TPCs in fourteen Apulian landraces, belonging to six legume species (fava bean, pea, chickpea, grass pea, common bean and lentil) was quantified. All the landraces were cultivated in their traditional environment during the growing season 2016-2017. TPCs of raw seeds were quantified. The amounts in methanol extracts, expressed as gallic acid equivalent (GAE), were measured using the Folin-Ciocalteau assay as modified by Lin and Lai [3].

The contents of TPC detected in the tested landraces are summarized in Table 1. On the whole, the fava bean landraces showed higher TPC values than the other species. The highest value was recorded for "Fava di Zollino". As expected, an appreciable variation of TPC amount among the landraces belonging to the same species was observed. The seed coat color is one of the reason that results in the TPC differences within each species. Pigmented seeds contain higher TPC than the light colored ones though coat represent approximately 10% of dry seed weight. We found that chickpea and grass pea landraces with colored coat ("Cece rosso liscio di Cassano delle Murge", "Cece nero liscio di Cassano delle Murge" and Cicerchia screziata della Murgia") showed higher TPC values as compared to the respective landraces with light-colored coat. The unexpected value recorded for "Fava viola", that, though colored did not have the highest TPC content among the fava bean landraces, could be explained by the large size of its seeds (100 seed weight: 205 g vs 125 – 142 g). This affects the coat/cotyledon ratio and consequently the TPC value of whole seed.

Finally, very similar TPCs values were recorded for the three pea landraces since all have light colored coat.
In general, the recorded values were within the range reported in the literature for the legume species object of the present investigation. For example, TPCs of fava bean from 3.7 to 9.5 mg GAE g^{-1}; pea ranged from 1.2 to 2.2 mg GAE g^{-1}; chickpea from 1.6 to 2.2 mg GAE g^{-1}; grass pea from 2.4 to 2.9 mg GAE g^{-1}; white common bean 2.0 to 3.2 mg GAE g^{-1} and lentil from 4.9 to 11.4 mg GAE g^{-1}. However, it should be taken in mind that the values reported in the literature refer to commercial varieties cultivated in different environments and years.

Legume species and landrace name	**TPCs (mg GAE g_{ss}^{-1})**
Fava bean	
Fava di Carpino	6.10 ± 0.314
Fava viola	7.24 ± 0.593
Fava di Zollino	10.73 ± 0.690
Fava barese	7.55 ± 0.275
Pea	
Pisello nano di Zollino	1.46 ± 0.093
Pisello riccio di Sannicola	1.75 ± 0.123
Pisello secco di Vitigliano	1.48 ± 0.136
Chickpea	
Cece bianco liscio	1.40 ± 0.079
Cece nero liscio di Cassano delle Murge	2.14 ± 0.102
Cece rosso liscio di Cassano delle Murge	1.97 ± 0.049
Grass pea	
Cicerchia bianca del sud-est barese	1.63 ± 0.043
Cicerchia bianca della Murgia	1.64 ± 0.110
Cicerchia screziata della Murgia	3.28 ± 0.117
Common bean	
Fagiolo monti Dauni	2.01 ± 0.010
Lentil	
Antiche popolazioni di lenticchia di Altamura	5.79 ± 0.314

Table 1. The TPC content of the Apulian legume landraces.

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ALTERNATIVE ANALYSIS METHODS FOR THE SAFFRON (*CROCUS SATIVUS* L.) PRODUCT CLASSIFICATION

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Saffron, the red dried stigmas of *Crocus sativus* L., is considered as one of the most expensive spice used in food industry, because its harvest is very laborious, the number of flowers per hectare is extremely low and its processing requires a very intense work. Hence, it is important to monitor the quality of saffron available in the market. The quality of saffron is determined according to the ISO 3632-1.2:2011 that classifies it into three categories with regard to a large number of physical and chemical parameters that define saffron quality: microscopic characteristics, presence of flower waste, moisture and volatile matter content, ash content, aqueous extraction and UV-vis spectrophotometric determination at 440 nm, 330 nm, 257 nm, etc. [1-2]. These last three parameters are historically related to the content of crocetin, safranal and picrocrocin esters, respectively.
Whit this work, we propose a new analytical method for the saffron product classification, more accurate and sensitive than the ISO 3632-1.2:2011 procedure. First of all, we compared the extractive potential of solvents such as water, methyl alcohol and ethyl alcohol and we identified a hydrolysis phenomenon for the components extractable from the wires and the saffron powder, during the maceration in water. Moreover, comparing the traditional maceration with the Naviglio extractor, in relation to the solvent used, it has been seen that there is the possibility to obtain an extract in absolute ethyl alcohol with unique characteristics in relatively quick times. Another innovation concerned the analysis of the water content of the saffron sample. The ISO procedure is based on weight loss after drying in an oven, but using Karl Fisher's method it could possible to obtain best results, with a good repeatability and good precision, in less time [3]. In addition, UV–vis spectrophotometric analysis are not sufficient to properly discriminate the quality parameters of saffron inasmuch $E_{1\ cm}^{1\%}$ of 440 nm is directly correlated with the ability to transmit colour to food and beverages, but the $E_{1\ cm}^{1\%}$ of 257 nm and $E_{1\ cm}^{1\%}$ of 330 nm do not give an accurate measurement of picrocrocin and safranal, because of existing interferences. With regard to picrocrocin determination at 257 nm, also the crocetin esters absorb at the same wavelength. The direct consequence is that the categories established by ISO/TS 3632-1.2:2011 related to $E_{1\ cm}^{1\%}$ of 257 nm (category I, minimum 70 units; category II, minimum 55 units; and category III, minimum 40 units) do not represent the real content of picrocrocin. Therefore, for its correct identification, it is preferable to use high performance

liquid chromatography (HPLC). Finally, freeze-drying allows saffron to be obtained with a very low moisture content and consequently a higher content of crocetins, a longer shelf life, a greater stability and a higher coloring power.

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DETECTION OF FATTY ACIDS PRODUCED BY SPECIES OF *NEOFUSICOCCUM*, FUNGI ASSOCIATED WITH GRAPEVINE TRUNK DISEASES

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Several fungal pathogens are involved in grapevine trunk diseases (GTDs). In particular, species of the family Botryosphaeriaceae, as Botryosphaeria, Diplodia, Lasiodiplodia and Neofusicoccum, have become an impending threat to productivity and longevity in most wine-growing areas by causing so-called Botryosphaeria dieback [1].
Diverse Botryosphaeraceae species were studied for the capacity to produce secondary metabolites as virulence factors [2, 3]. In this respect, there are few information on *N. vitifusiforme*. A strain of *N. vitifusiforme* with high pathogenicity *in planta* has been isolated from grapevine in Sicily [4] and preliminary *in vitro* studies on its culture organic extract showed the presence of fatty acids. Considering that, fatty acids and modified fatty acids are important compounds during the colonization of plants by pathogenic fungi and may be involved in their virulence, qualitative and quantitative analysis were conducted to determine the fatty acids composition. Furthermore, analysis on fatty acids produced by *N. parvum* were conducted on a strain which produce *in vitro* essentially naphthalenone polyketides [5].
Fatty acids present in the culture organic extracts of *N. vitifusiforme e N. parvum* were identified and quantified via essentially GC-MS after esterification with diazomethane in ether.
Interesting results were obtained from *N. vitifusiforme*. In fact, five different fatty acids and one dicarboxylic acid were detected in its crude extract. The most abundant component identified is linoleic acid (48.2%). Octadecenoid acids have influence in the fungal virulence because many of them are precursors of jasmonic acid, a plant hormone capable of inducing phytotoxic effects.
Even if in low concentration, *N. vitifusiforme* also produces azelaic acid, a dicarboxylic acid well-known for its bacteriostatic and bactericidal proprieties against diverse microorganisms.

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MULTI-TARGETS MRM MASS SPECTROMETRY APPROACHES FOR METABOLOMIC INVESTIGATIONS

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Metabolomics is having a great impact on numerous disciplines during the last years, offering to scientists the privilege to acquire a comprehensive and detailed knowledge of molecular composition of any biofluid [1] or food [2].
Thanks to its significantly higher sensitivity and fast data acquisition, MS plays a dominant role in the metabolomics field for investigation both in clinical applications or in several areas related to food sciences e.g. to quality control along the production chain from raw material to finished product, to food frauds or to nutrition.
MS is intrinsically a highly sensitive method for detection, quantitation, and structure elucidation of upwards of several hundred metabolites in a single measurement.
This work presents new multi-targets methods based on multiple reaction monitoring/mass spectrometry (MRM/MS) approach for phospholipids, oxylipins, catechins and flavanols quantification.
Our attention was focused on molecule classes highly interesting for clinical investigations and quality control and food composition following a simple extraction protocol e.g. solid-liquid or liquid-liquid combined to an optimized LC-MS/MS in MRM ion mode analysis as reported in Figure 1.

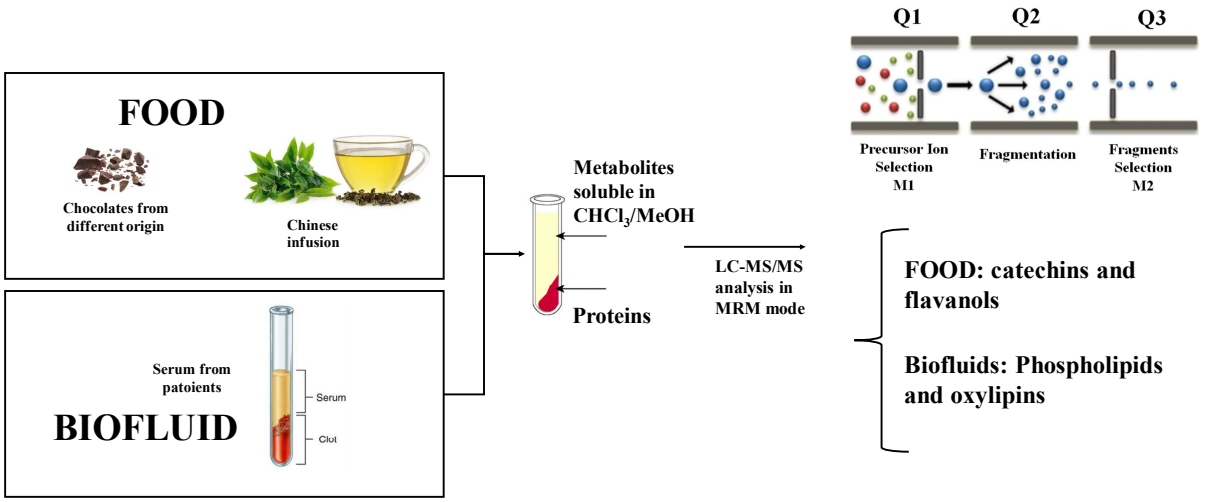

Figure 1: experimental workflow for LC-MS/MS analysis on different matrices

Our work presents new multi-targets methods based on MRM/MS approach for phospholipids, oxylipins for clinical apllications and catechins and flavanols quantification for food investigations.

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For clinical purposes our attention was focused on molecule classes highly interesting for their crucial physiological role e.g. phospholipids and oxylipins. The quantification of phospholipids including phosphatidylcholine, phosphatidyletanolamine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol and alkylacyl-phosphatidylcholine and related lyso-forms was resulted to be useful for picking up potential biomarkers of Celiac disease metabolism within the panel of more than 50 phospholipids monitored in a single analysis. Another class of molecules was related to oxylipins for profiling the inflammatory mechanisms in patients affected from systemic sclerosis by the simultaneous, sensitive, fast, and reproducible measurement of 80 oxylipins in a single MRM run for clustering the state of pathology at a different level of inflammation.

From the nutritional viewpoint, the plants are the main source of secondary metabolites with great antioxidant functions produced as a defense mechanism. Our study was focused on the optimization of a LC-MRM/MS method for the quantitative determination of polyphenolic component of various chocolates from different countries. The comparison of profile of 57 polyphenols in all the chocolate samples has highlighted the different content of such metabolites strongly correlated to the percentage of cocoa powder used for manufacturing. The main result of our study was that the Ecuador chocolate contained the highest content of the main polyphenols characterizing the chocolates.

Further, 62 analytes were selected for targeted analyses of Chinese teas including both green and black infusions with a different profile in agreement with the level of fermentation of original herbs. Epicatechin and catechin were resulted to be more abundant in green teas than the other tea infusions whereas fermentation process occurring during the manufacturing of black and oolong tea significantly affected the catechins level. The gallic acid was really abundant in black infusions jointly to the its derivates, e.g. ethyl gallate and procyanidin dimer gallate. The higher level of gallic acid recorded in black tea than green infusion was due to the fermentation process that remarkably increased its content.

In conclusion, we have developed method for identifying and quantifying species of different nature in a single chromatographic run, exploiting the potential of the LC-MS/MS in MRM ion mode to obtain high sensitivity (few μg/L for all target molecules) and reproducibility of the data (CV% that can vary between 5 and 15%).

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UNTARGETED METABOLOMIC ANALYSIS OF SNAIL SLIME BY ULTRA-HIGH RESOLUTION MALDI-FT-ICR MASS SPECTROMETRY

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Chronic wounds result from the failure of the normal wound healing process. [1] Any delay during the tissue repair process could be defined as chronic wound healing and can have a highly detrimental impact on human health. To face this problem, utilization of nanotechnology and natural products is gaining more and more attention, in order to avoid synthesized therapeutic agents, the utilization of whom can cause undesired side effects. In detail, snail slime products and gold nanoparticles (AuNPs) are increasingly being used for skin injury therapeutic purposes [2, 3], thanks to their ability to accelerate tissue repair process. However, common synthetic routes for AuNPs involve utilization of organic solvents and production of toxic by-products and this limits their utilization for medical purposes [4]. Thus, it's really important to find "green" ways to synthesize AuNPs that involve utilization of natural products and to understand which kind of species actively take part to the synthesis and product stabilization. In this work, a metabolomic approach was assumed in order to characterize metabolic profile of a sample of pure snail slime and another one obtained by the synthesis of AuNPs by reduction of HAuO4 conducted using pure snail slime as solvent and to obtain insights on which kind of metabolites actively takes part to the reaction. To achieve this aim, Ultra-High Resolution Mass Spectrometry was used and obtained data were used to perform a rapid analysis of metabolome by converting accurate m/z values in putative elemental formulas in order to better understand the chemical composition of the sample. Molecular formula maps were obtained by making 2D Van Krevelen plots, that lead to a direct identification of different classes of metabolites [5]. In this way, the presence of important classes of metabolites, i.e. fatty acid derivatives, amino acids and peptides, carbohydrates and polyphenolic compounds, could be appreciated in both kind of samples. Moreover, direct comparison of Van Krevelen plots suggests that sulphur-bearing amino acids and peptides mostly takes part to the synthesis and stabilization of AuNPs, thus supporting the idea of snail slime as perfect as their carrying-agent.

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APTAMERS TRUNCATION BY SECONDARY STRUCTURE ANALYSIS AND THEIR APPLICATION TO DETECT GLIADIN

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Aptamers are short single-stranded oligonucleotide sequences selected in vitro through a process called Systematic Evolution of Ligands by EXponential enrichment (SELEX) to bind a specific target. This process is characterized by the screening of a large library of different DNA sequences, to isolate and identify functional molecules. The length of selected aptamers (typically 30-60 bases) can affect their affinity, due to the presence of nucleotides not involved in the interaction with the target. Therefore, the identification of binding domains and truncation of these selected sequences is a critical step to obtain aptamers with higher affinity and with lower production costs. Aptamers fold into unique structures that usually include stems and loops. These structures are central to target molecule recognition and any disruptions result in poor binding abilities. If non-essential nucleotides are eliminated by truncation, the aptamer may adopt conformations that resulted in a stronger aptamer-target complex.
We are pioneers in performing the SELEX process to obtain aptamers against the immunotoxic peptide of gluten 33-mer, not only in aqueous buffer [1], but also in deep eutectic solvents (DES-SELEX) [2]. The two aptamers resulting from the selective process, Gli1 and Gli4, have been subjected to a truncation study in order to improve their analytical performances. On the basis of their secondary structure evaluated in silico, two shorter aptamers have been designed and investigated. The dissociation constants (K_d) between aptamers and the target molecule were determined by means of a labelled-based assay onto magnetic particles (MPs) with chronoamperometric detection. Lastly, a sandwich-type assay for the detection of gluten was developed. According to the results of the present study, the truncated aptamer Gli4trunc1 can be used to capture and detect gliadin.

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A RECOMBINASE POLYMERASE AMPLIFICATION - LATERAL FLOW ASSAY FOR THE ONSITE RAPID DETECTION OF THE EMETIC *BACILLUS CEREUS*

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Bacillus cereus is increasingly recognized as the etiological agent of gastrointestinal and non-gastrointestinal diseases. Two clinical pictures connected to food poisoning, diarrhea and emesis can be distinguished. Heat-labile enterotoxins elicit diarrhea, while a heat-stable depsipeptide toxin, called cereulide, provokes emesis. In general, both types of food-borne disease are relatively mild and self-limiting. Nevertheless, during the last few years, severe forms of disease caused by emetic B. cereus have occasionally involved hospitalization or even death [1]. Due to the increasing number of reports of food-borne disease, especially of severe cases, fast detection methods are required for diagnostic purposes as well as for the prevention of food contamination and food-borne outbreaks.

In case of foodborne outbreak, the investigation relies on traditional microbiological methods, often supported by molecular assays for the detection of the genetic determinants of the emetic toxin. Recently, real-time polymerase chain reaction (RT-PCR) has been proposed as a highly specific and sensitive tool for food-borne pathogens detection furthermore offering the potential for quantification. Still, the RT-PCR method is not suitable for the rapid and onsite detection. Recombinase polymerase amplification (RPA) is a novel isothermal technology that is increasingly used to improve the rapidity and sensitivity of molecular diagnosis of foodborne pathogens. The RPA technology, however, requires a detection method of the amplified product. Therefore, we developed a RPA for cereulide-producing Bacillus cereus using a Recombinase Polymerase Amplification assay based on the sequence of the cereulide synthesis gene, and combined it to a visual Lateral Flow Assay (LFA) as a portable and rapid detection tool.

Cereulide-producing B. cereus NCTC 11143 and Cereulide-non-producing B. cereus ATCC 14579 were used as reference strains. Specific RPA primers targeting ces genes were designed with Primer3Plus software, according to TwistDx guidelines.

For LFA visualization, the forward and reverse primers were modified with 6-Fam and biotin at the 5' end, respectively. The LFA device was fabricated by coating streptavidin at the Test line, while an anti-fluorescein antibody was labelled with gold nanoparticles (GNP). In the presence of the amplified gene, the biotin-tag allowed its capturing at the Test line, while the FAM-tag reacted with the GNP-labelled antibody. In conclusion, a visible red line, due to the surface plasmon resonant band of GNP, was visible at the Test line for sample containing Bacillus cereus [fig.1].

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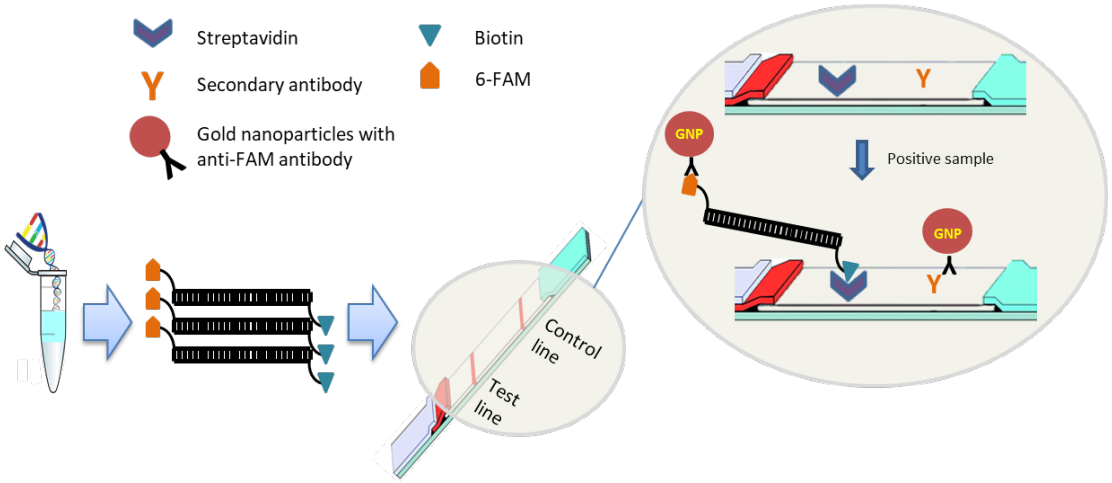

Figure 1. Schematic of the RPA-LFA: DNA from B. cereus is extracted, isothermally amplified and the amplified product double labelled with biotin and FAM revealed by a visual lateral flow assay employing streptavidin as the capturing reagent and an anti-FAM antibody linked to gold nanoparticle as the probe.

The RPA in optimal conditions allowed for the specific amplification of the positive strain in 20 minutes at 37°C. The amplified product was loaded into the LFA device after 1/100 dilution and the visual result recorded after 5-10 minutes. The RPA-LFA allowed for detecting a positive reaction with < 6 fg DNA per reaction. No false positivity was observed for the negative strain. Moreover, the novel RPA-LFA was applied to detect B. cereus in model contaminated food, such as cooked potatoes and rice, confirming the ability of the assay as a reliable and rapid tool for food safety monitoring.

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NOVEL DESIGN FOR GRAPHENE-BASED WATER-GATED FIELD-EFFECT TRANSISTOR FOR SENSITIVE BIOSENSING

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In the recent years, electrolyte gated organic field effect transistors (EGOFETs) have gained a leading position among biosensors in terms of sensitivity and operation time.[1] Fast and ultra-sensitive responses are reported in literature for a wide range of biological analytes, spacing from clinical diagnostic to food analysis. These devices still suffer of some reproducibility issues due to the organic nature of the employed semiconductor. In this context, a promising material for the realization of high-performance, cost-effective and reproducible sensors is graphene.[2] The typical approach for graphene based EG-FETs architectures is to bind the bio-receptor onto the graphene surface, usually using weak interactions like π-π stacking.[5]

In this work, a novel design for electrolyte-gated FETs biosensors based on graphene is proposed. The functionalization with a bioreceptor (anti-IgG) occurs on the gate electrode in place of the conventional channel functionalization. We have recently demonstrated that functionalizing the gate with an opportunely designed self-assembled monolayer (SAM) containing the bio-receptor, is a good strategy to reach the sensitivity limit of the single-molecule detection.[8,9] Actually, the presence in the SAM of an extended network of hydrogen bonds, can act as amplifier of the analyte-receptor interaction. The binding event generates a dipole moment that induces a re-arrangement of the H-bond network, allowing in this way to sense also a very limited number of binding events. This approach presents several advantages: i) the bio-functionalization of the gate is simpler and more stable and reproducible as it relies on the formation of a covalent binding between the Au surface and the SAM; ii) the SAM can play a key role in the sensing process, leading to an amplification of the signal generated by the binding event; iii) graphene represents a more robust alternative as compared to organic semiconductors; iiii) graphene is not directly involved in any functionalization reaction; iiiii) it offers the possibility to perform the incubation with the analyte solution outside from the EG-FET structure. This last aspect allows the use of deionized water as gating electrolyte; the low ionic strength prevents Debye screening

effects. Using this approach, a limit of detection (LOD) of about 100 aM was found and wide dynamic range was observed. Cross-reactivity towards IgM untargeted molecule was very limited, even in the nanomolar range. This new design for a graphene-based EGOFET allowed to achieve a selective biosensor for IgG with a sub-femtomolar sensitivity.

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DEVELOPMENT OF BIOCHAR-BASED SCREEN PRINTED ELECTRODES: TOWARD A NEW MATERIAL FOR ELECTROCHEMICAL SENSOR

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Biochar is a carbonaceous material obtained from the pyrolysis of vegetable organic material that is able to improve agricultural productivity, clean up contaminated land, and to reduce the impact of climate change by promoting carbon sequestration.
In this paper the electrochemical properties of screen printed electrodes modified with biochar (Biochar/SPE sensor), prepared with the modification of SPEs by drop casting with a stable dispersion of biochar, have been reported. This study was conducted using different electroactive species, such as ferricyanide, benzoquinone, epinephrine, ascorbic and uric acid, in order to understand the electrochemical behaviour of the modified electrode. The results were compared with those of commercial screen-printed electrodes confirming that modification allowed to obtain a sensor with improved electrochemical behaviour in terms of resolution, peak-to-peak separation, current intensity, and the resistance of charge transfer. A tyrosinase biosensors (Ty/Biochar/SPE) has been developed using the Biochar/SPE for the determination of epinephrine. The enzyme has been immobilized onto the working electrode of SPE, after the modification with Biochar, by cross-linking with glutaraldehyde. The detection has been performed by measuring the cathodic current due to the reduction of the corresponding quinone [1] at low potential, equal to -0.310 V for epinephrine. The experimental conditions for the tyrosinase immobilization and the analytical parameters, such as applied potential and pH of buffer have been studied and optimized. Under these conditions, the electrochemical biosensors have been characterized. A linear working range of epinephrine was obtained from 0.05 up to 0.5 mM. The detection limit is 2×10^{-4} mM for developed biosensors. The biosensors construction was highly reproducible.

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PAPER-BASED ELECTROCHEMICAL (BIO)SENSORS FOR SUSTAINABLE SURFACE WATER MONITORING

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The paper-based colorimetric assays have been widely reported in literature being cost-effective, not requiring additional components (i.e. pump) for microfluidic handling of the solution and avoiding the sample treatment thanks to the filtering property of the paper. In the last decade, the electroanalysis has discovered the utility paper as electrode-active support, converging the reported advantages of paper with the features of electroanalysis such the high sensitivity, selectivity and the capability to work in complex matrices (e.g. coloured samples) [1]. Herein, we described the novel reagentless and sustainable paper-based electrochemical (bio)sensors, manufactured with a simple and inexpensive approach for pollutant detection in surface water. 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 pollutants in standard solutions and in real samples (river water). This novel and highly sustainable configuration were designed 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 [2]. The filter paper has been also combined with the butyrylcholinesterase enzyme (BChE) for the detection of pesticides in rivers and waste waters. The principle of this approach is based on dual parallel electrochemical measurements 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 [3]. Beside the filter paper, also the office paper, with different rheological properties, has been exploited as substrate to print the electrode developing novel origami paper based-biosensors for a multifarious detection of three classes of pesticides namely organophosphorus, triazine and organochlorine compounds. In detail, we developed an amperometric biosensing tool constituted of three paper-based biosensors based on the inhibition of three different enzymes namely: butyrylcholinesterase for the detection of paraoxon (an organophosphorus insecticide), tyrosinase for the quantification of atrazine (a triazinic compound), and alkaline phosphatase for the measurement of 2,4 dichlorophenoxyacetic acid (a synthetic auxin). To deliver a fast and easy measurement, all the required reagents are loaded on the paper-based patterns; thus, the operator needs

only to add 5 µL of the real samples to carry out the measurement. This paper-based biosensing tool was successfully challenged in standard solutions as well in river water samples achieving detection limit at ppb levels [4]. Furthermore, herein we reported a novel flower-paper based device encompassing four different paper-based inhibitive biosensors for allowing the detection of four types of pesticides including glyphosate, which is a relevant pollutant in surface water.

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AN ORIGAMI PAPER-BASED LAB-ON-A-CHIP FOR PRECISION MEDICINE IN ALZHEIMER DISEASE

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The rapidly aging of the population has increased the number of patients affected by several pathologies correlated to the aging, including the neurodegenerative disorders such as Alzheimer and Parkinson diseases. Consequently, the last decades have seen an increase of higher mortality as well as morbidity rates and healthcare costs for treatment, hospitalization and care assistance for this type of disorders. To this regard, the cost for managing patients affected by neurodegenerative disorders has been estimated to be approximately € 130 billion/year [1]. The recent trends of precision medicine have boosted the personalization of medical care in several field including neurological diseases, thanks to new diagnostics and therapeutics developed and discovered, respectively [2]. In this overall scenario, we propose a novel paper-based lab-on-a-chip for to deliver a cost-effective and easy to use sensing tool for a customised administration of drugs for Alzheimer disease. Among several drugs, we have designed the device for evaluating the efficiency of alkaloid compounds (e.g. Physostigmine), used for Alzheimer disease treatment. These compounds are employed being able to inhibit in reversible way the cholinesterase enzyme. Since the activity of cholinesterase is different among the patients, the administration of the customised amount of drug can improve the treatment and the quality of patient life, avoiding side effects due to the overdosage. Herein, we propose a paper-based device to measure the cholinesterase activity in blood for evaluating the efficiency of Physostigmine inhibition. In detail, we exploited office paper to print the electrode and VividTM Plasma Separation membrane to threat the blood sample as well as to load the reagents needed for the measurement, delivering a reagent free analytical tool. For cholinesterase activity measurement, butyrylthiocholine was used as enzymatic substrate and the by-product thiocholine was detected by using an office-paper screen-printed electrode modified with Carbon Black and Prussian Blue nanocomposite [3]. The use of this nanocomposite relays on its capability to electrocatalyse the oxidation of thiocholine allowing its detection at low applied potential (+300 mV vs. Ag/AgCl pseudoreference), without any fouling problem occurring at bare electrodes. For enzymatic measurement, several parameters have been optimised such as the concentration of substrate (i.e. 100 mM) and time of reaction (6 min), taking into account the sensitivity and the repeatability of the analysis. The calibration curve obtained in real blood sample gave a linearity between up to 12 U/mL with a sensitivity of 0.050 ± 0.004 μA mL/U. The results obtained measuring the Physostigmine inhibition activity against cholinesterase enzyme in matrix will be reported.

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A SENSITIVE ELISA METHOD FOR THE DOSAGE OF INSULIN GLARGINE® IN RAT PLASMA AND SERUM TO SUPPORT "IN-VIVO" PRE-CLINICAL TRIAL MONITORING.

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Nowadays, the people who live with type-1 diabetes are estimated to be about 400 millions worldwide. Diabetes mellitus is a very well-known chronic disease due to a dysfunction in the equilibrium of the pancreatic hormone insulin. Diabetic people manage their condition taking subcutaneous injections of insulin twice or thrice a day forever. Subcutaneous administration of insulin in people with diabetes is associated with the distress of daily injections, with implications on quality of life. Therefore, innovative therapeutic approaches for glucose control through oral administration of insulin have revolutionary potential, both for patients and industry, disclosing new perspectives for the management of type 1 diabetes. Insulin-loaded SLN (Solid Lipid Nanoparticles) formulations have been obtained and their chemical and biological activity preservation has been verified by in-vivo studies [1]. Further investigations have demonstrated that an insulin analogue (i.e. insulin glargine® that is a DNA recombination modified version of the traditional insulin able to cover the whole day by a single take [2], [3], [4]) is particularly suited for incorporation in SLN and for its subsequent resuspension. However, no analytical tools for the rapid and efficient monitoring of insulin glargine® are available. The aim of the project was to develop an immunoenzymatic assay method to follow the in-vivo pre-clinical trial of the new drug formulations through the dosage of insulin glargine® in plasma and serum of treated rats. We developed and in-house validated by determining LOD (0,8 ng/mL), Dynamic Range (1,2 - 20 ng/mL), Accuracy (CV%) and Selectivity (Cross Reactivity <2% to other forms of insulin) of the assay, which was demonstrated to allow measuring insulin glargine® at levels required for the pre-clinical study and correlated with glycemia measurement. Among other advantages, the assay requires as low as 20µL of serum sample and is completed in 3 hours.

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DEVELOPMENT OF A MULTIRESIDUE SCREENING METHOD BASED ON PULSED AMPEROMETRIC DETECTION AT A GLASSY CARBON ELECTRODE FOR SULPHONAMIDES MONITORING IN FOODSTUFFS OF ANIMAL ORIGIN

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Sulphonamides (SAs) are antimicrobial drugs possessing a significant chemotherapeutic activity against infections caused by gram-positive and gram-negative bacteria and some protozoa. The widespread and often uncontrolled use of SAs in veterinary practices can contribute to their potential increasing presence in farm animals [1], and, as a consequence two major adverse impacts on human health: bacterial resistance [2] and toxicological effects resulting from their residues in food [3]. In order to increase the food safety, the European Union has established a maximum residue limit (MRL) of 100 μg kg^{-1} for the sum of all SAs in milk, tissues and muscle [4]. In the last years, several confirmatory methods were proposed for the monitoring of SAs in foodstuffs of animal origin, based on liquid chromatography (LC) separations coupled with mass spectrometry, UV-DAD and fluorescence detectors [5-8]. In addition, owing to the large number of samples to be processed, screening methods were developed, mainly immunoassay-based techniques [9-10], that usually are cost effective, due to the need of specific biocomponents, and show high false positive rates. In the light of these findings, an alternative screening method based on constant potential amperometry at a glassy carbon electrode (GCE) was proposed [11]. Nevertheless, the detection at GCE was proven not useful for organic compounds, whose oxidation at the electrode determines a strong electrode fouling with a consequent time dependent deterioration of the electrode response [12].
On the basis of the above findings, the purpose of the proposed research was the development of a screening method based on an effective pulsed amperometric detection (PAD) at a GCE tuned with LC separation, suitable for a multiresidue quantitative screening of SAs in foodstuffs. Preliminary experiments have been carried out by cyclic voltammetry to investigate the SAs electrochemical behaviour in mobile phases at different composition, and to select the waveform detection and cleaning electrode potentials. Flow injection analyses with SAs standard solutions have been performed to optimize the PAD waveform, which revealed excellent reproducibility, sensitivity and response stability characteristics in comparisons with those obtained by constant potential and integrated pulsed amperometric detections. In order to comply with requirements of screening methods the optimized electrochemical detection system has been coupled to a fast and efficient LC separation method [13]. The optimized LC-PAD screening method showed excellent chromatographic (peak symmetry, peak width, resolution) and performance (sensitivity, limits of detection and quantification, linearity) parameters. For all the SAs investigated the linearity was in the range 50 –

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500 µg L^{-1} (r^2 in the range 0.9948 – 0.9981) while the operational range was extended until 2 mg L^{-1}. PAD also allowed very low detection (in the range 2 – 3 µg L^{-1}) and quantification (7 – 9 µg L^{-1}) limits to be achieved. Performances of the proposed quantitative screening method have been also tested by comparing chromatograms (see Fig. 1) of a blank milk sample, a spiked blank milk sample at 10 µg L^{-1} and a standard mix of SAs at 10 µg L^{-1}.

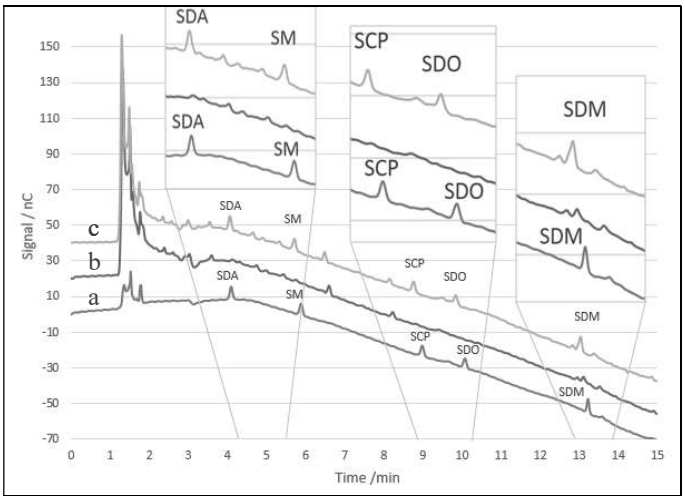

Figure 1. Chromatograms of a standard mix of SAs at 10 µg L^{-1} (a), a blank milk sample (b), and a spiked blank milk sample at 10 µg L^{-1} (c). Sulfadiazine (SDA), sulfamerazine (SM), sulfachloropyridazine (SCP), sulfadoxine (SDO), sulfadimethoxine (SDM).

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POLYPYRROLE FILM DEPOSITION ON NANOPOROUS SILICON (PSi) INTERFEROMETERS FOR THE DEVELOPMENT OF MIP-BASED OPTICAL SENSORS

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Polypyrrole (PPy) is a thermoplastic and chemically stable polymer in room temperature [1]. The simplicity of the synthetic procedures and availability of the monomers are attractive features of PPy. Commonly available processes to fabricate polypyrrole are chemical and electrochemical oxidation [2,3]. Aqueous or anhydrous $FeCl_3$, other salts of iron (III) and copper (II) are widely used as chemical oxidants. Thickness and features of the resulting PPy films are affected by a variety of factors, among which are the choice of solvent and oxidant, initial pyrrole/oxidant ratio, reaction time and temperature [2,3]. Preparing thin films is crucial when PPy is used in sensing applications as this allows an easier integration with the transduction surface. The use of PPy film has been reported for the development of electrochemical [4,5] sensors and for optical sensors [2,4] for detecting humidity, hydrogen (H_2), nitrogen dioxide (NO_2), ammonia and organic vapours [2]. PPy can be exploited as an excellent tool for the preparation of selective systems through the molecular imprinting method [4,6]. It is based on forming polymeric structures in presence of a template molecule. In the synthesis of Molecularly Imprinted Polymers (MIPs), monomers are polymerized around template molecules. After the removal of template molecules from the polymeric structure, they leave complementary cavities to their three-dimensional structures. MIPs can be thus used as selective recognition elements, that mimic the recognition ability of bioreceptors, but with greater characteristics of resistance and stability [7]. Molecularly Imprinted Polypyrrole (MIPPy) has been integrated into sensors for the detection of toxins, metabolites, antibiotics, pollutants and proteins [2,3,4] mostly using an electrochemical transducing mechanism, with less attention to optical sensors, even though they usually provide excellent sensitivity [2]. In particular, no examples of PPy or MIPPy integration with optical transducers such as nanoporous silicon (PSi) interferometers are reported so far. PSi has emerged as an attractive nanomaterial for the design of optical devices, owing to its large surface area, versatile chemistry and straightforward fabrication. In PSi-based interferometers, a change in refractive index of the solution contained within the porous nanostructure can be measured by visible reflectance spectroscopy. The reflectivity spectrum from porous Si is governed by the Fabry-Perot relationship: $m \lambda = 2nL$, where m is the spectral order of the optical fringe, λ the wavelength, n the refractive index of the film, and L its thickness. The product nL is the quantity referred to as “effective optical

thickness" (EOT) and is obtained by Fourier transformation of the reflectivity spectrum. Any change of the refractive index n will induce a proportional shift of the position of the interference fringe position λ. Thus, the sensing concept relies on monitoring changes in the EOT of PSi as a response to target binding [8] achieving selectivity by its integration with a selective material.

Herein, we propose for the first time new methods to combine the high selectivity of MIPPy films with the optical properties of PSi, with the goal of developing highly selective and sensitive optical sensors for the detection of targets of biomedical and environmental interest. Thin films of PPy were deposited on surface of PSi interferometer by in situ chemical polymerization through two different synthetic approaches: vapor- and liquid-phase deposition. The first one consists in the incubation of PSi in $FeCl_3$ 1.5% w/v in EtOH (20 min) and subsequent insertion in a chamber filled with pyrrole-saturated air at R.T., for different time intervals (1h, 5h, 8h and overnight). The second one consists in the incubation of PSi in $FeCl_3$ 0.1 M (50 min) and subsequently in a 0.1 M pyrrole solution for different time intervals (1h, 5h, 8h and overnight). PPy deposition is monitored by visible reflectance spectroscopy evaluating EOT values before and after PPy deposition on the porous layer. Preliminary results suggest that PPy vapor phase deposition determines a more homogeneous film deposition as well as higher EOT values for each tested polymerization interval possibly corresponding to higher thickness of resulting film.

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A MICROFLUIDIC SETUP MADE BY AN ION EXCHANGE MICROCOLUMN COUPLED WITH AN ELECTROCHEMICAL SENSOR FOR ON-FIELD DETERMINATION OF HEAVY METAL IN WATER MATRICES

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As a kind of persistent pollutants, heavy metal ions represent a source of environmental contamination due their toxicity and accumulation in the environment. Nowadays, monitoring of these pollutants is obtaining attention and a wide range of chemical sensors and biosensors were proposed by literature for the determination of heavy metals. Compared to traditional methods, electrochemical measurements offer versatility, low cost and user-friendly applications. In the field of biosensors, the enzymatic inhibition by heavy metal ions has been proposed the basis for gross sensors to evaluate this contamination in complex environmental matrices. While application of biosensors can give an integrated response from heavy metals, perhaps in relation to their bioavailable fraction, selectivity is quite low, and speciation cannot be obtained as enzymatic inhibition is not so specific. Therefore, the selectivity should be the result of a preceding separation of different ionic species from complex matrices [1], preferably in line with detection. The development of such a system for on field detection is highly desirable.
In this work we present a microfluidic setup for the simultaneous separation and detection of metal ions in water samples. A miniaturised glass column packed with ion-exchange resins has been coupled with a previously optimised inhibition biosensor based on glucose oxidase (GOx) for the electrochemical detection of heavy metals [2]. The influence of different experimental variables on retention of several metal ions was optimised.

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DEVELOPMENT OF A PAPER-BASED BIOSENSOR FOR THE DETECTION OF URINARY PROSTATE SPECIFIC ANTIGEN (PSA)

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Prostate cancer represents the most common pathology and the second cause of tumour-related death for men worldwide [1].
Early diagnosed localized disease can be successfully cured by radical surgery or radiation; however, the majority of locally advanced and all metastatic diseases are treated with androgen deprivation. Despite an initial control of the disease with hormone therapy, later on it inevitably progresses to metastatic castrate-resistant prostate cancer, very difficult to cure [2]. Thus, an early detection is essential for the successful clinical treatment of prostate cancer.
Nowadays routine prostate-specific antigen (PSA) screening, combined with digital rectal examination and prostate biopsies analysis, are commonly used to detect prostate cancer. However some of these methods are invasive and non-precise to detect the pathology. The use of serum PSA for example can lead to overdiagnosis and overtreatment of prostate cancer resulting in controversy about its use for screening. Serum PSA also has limited accuracy in predicting outcomes after treatment and in making clinical decision about adjuvant and salvage therapies.
Since it is impossible to distinguish over-diagnosed tumours from others, the majority of patients found positive during the screening are offered surgery which is often accompanied by adverse effects (such as erectile dysfunction, urinary incontinence, infections) and strongly impacts the quality of life.
In addition to the inconvenience for the patients, the economic impact of such a high percentage of unnecessary tests on health service resources should be considered.
Therefore, novel clinically useful biomarkers are urgently needed to improve identification of men at risk and to predict the natural progression of the tumour [3].
In the past decades, different combination of urinary biomarkers (Interleukins, cytokines, chemokines etc.) were evaluated with the aim to provide more reliable results. Among them, urinary PSA or urinary to serum PSA ratio seem to be a useful biomarker to improve the diagnosis accuracy [4]. Moreover the sample collection is non-invasive.
A point-of-care test to detect urinary PSA could allow an easy and affordable way to screen the whole male population at risk.

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In this communication, the development of a paper-based biosensor for the detection of urinary PSA will be presented.

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HUMAN CHEMOSIGNALS ELICITED FROM EMOTIONAL STATES

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In the animal kingdom most species secrete or excrete chemical factors that trigger a social response in members of the same species [1]. Smell provides peculiar evolutionary advantages over vision and hearing, as it works even when other senses are impaired (e.g. in the dark or during sleep) and it allows obtaining information over long distances about the possible presence of a predator or a potential sexual partner. If olfactory communication is so important for animals, what about human race?
As a social species, humans rely on social interaction for their well-being and survival: are we unconsciously using olfactory clues to select our social relationships, choose a partner or communicate emotions like pain, fear or happiness? Literature does not provide any definitive answer to such questions, but more and more hints are cumulating which seem to suggest the exchange of chemical messages among humans [2].
The European Commission has recently funded the POTION project (Promoting social interaction through emotional body odours, FETPROACT-01-2018, Contract n° 824153) to investigate the nature of chemosignals and their sphere of influence on social interaction.
From the analytical point of view, the identification of human pheromones is impressively challenging. It is probable that such volatile or semivolatile chemicals are released to very low concentration levels, thus requiring a pre-concentration step before the analysis. Furthermore, it is also likely that distinctive odours consist of a cocktail of multiple chemicals, more than of a single compound, and the concentration pattern might be important. Finally, these odour components might belong to different chemical classes and have different properties, so that they may not be all detected with just a single method.
In this work, an analytical strategy for untargeted analysis is proposed to approach the problem. Risks and limitations are discussed together with possible solutions and applications in the clinical field.

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EVALUATION OF BIOMARKER STABILITY WITHIN A PROJECT FOR THE DEVELOPMENT OF AN INTEGRATED LAB-ON-CHIP AND POINT-OF-CARE DEVICE FOR NON-INVASIVE DIAGNOSIS AND THERAPY MONITORING OF HEART FAILURE PATIENTS (KARDIATOOL PROJECT-H2020)

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Heart failure (HF) is a rapidly diffusing chronic cardiovascular disease and one of the main causes of mortality and poor quality of life in western societies, for which early detection and continuous monitoring play a crucial role for tits prognosis and therapeutic treatment. The H2020 KardiaTool Project is aimed to develop Lab-on-a-Chip (LoC) and Point-of-care (PoC) devices as an innovative approach for the non-invasive, rapid and accurate determination of HF biomarkers in saliva samples. Saliva is a promising biological fluid alternative to blood, urine or tissues that are conventional specimen to assess patients' health status. Saliva can be easily and unobtrusively collected, even from critical subjects (e.g. children, elder and disabled people [1,2]. The KardiaTool LoC-PoC devise will allow to monitor four different HF biomarkers, namely N-terminal pro-brain natriuretic peptide (NT–proBNP), tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10), and cortisol in saliva samples [3-4]. Currently, the determination of cortisol in saliva is performed by analytical methods such as chromatography, mass spectrometry and iphenated techniques, but NT-proBNP, TNF-α and IL-10 are usually determined only in blood, plasma or serum by immunochemical methods (e.g. enzyme-linked immunosorbent assay, ELISA). Several commercial ELISA kits are available for plasma, serum or urine analysis, but their usability has not reported for saliva analysis. At the same time, the concentration of analytes in saliva can be affected by sample storage, which thus have to be investigated in order to translate a saliva biosensor from a laboratory-proven concept to a reliable LoC-PoC device.
In this study we investigated both ELISA kit practicability to saliva analysis and the effect of storage conditions to define the best approach in the development of the KardiaTool LoC-PoC. Commercially available ELISA kit intended for cell culture supernates, serum, EDTA plasma, heparin plasma, and citrate plasma have been validated for the quantification of NT-proBNP, TNF-α and IL-10 in saliva. Matrix effect, reproducibility, repeatability, and sample recovery were evaluated. In addition to ELISA kit validation for salivary NT-proBNP, TNF-α and IL-10 quantification, an ultra-high performance liquid chromatography coupled to electrospray ionization-tandem mass spectrometry (UHPLC-ESI-QQQ) method was

developed for salivary cortisol determination. Finally, both short-term and long-term stability studies were carried out.

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A SAMPLER PROTOTYPE FOR THE SIMULTANEOUS COLLECTION OF EXHALED BREATH AND EXHALED BREATH CONDENSATE

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Exhaled air and breath condensate contain many health biomarkers, such as volatile and semi-volatile organic compounds, proteins and lipids. The potential relationship between these chemicals and specific diseases makes breath analysis a powerful tool of modern medicine for the non-invasive monitoring of health conditions. Due to the different etiopathogenesis of human diseases, only the monitoring of both the volatile and not volatile fraction of breath components may guarantee an adequate sensitivity and specificity for clinical applications.
Nowadays, the collection of breath samples is carried out by commercial or lab-made sampling systems that collect only one type of sample (e.g. gaseous or condensate phase), thus limiting the diagnostic capability of breath tests.
In this work, we present a portable prototype optimized for the simultaneous collection of gaseous exhaled breath and exhaled breath condensate within five minutes, allowing a comprehensive characterization of the subject. The system was optimized to minimize pressure drop and contamination of breath samples as well as to maximize the subject's comfort. The prototype was preliminary tested by collecting samples from healthy volunteers, and ethanol, isoprene, acetone, isopropyl alcohol, 1-propanol, 2-butanone, 2-pentanone, toluene and xylenes, and cortisol and 8-iso-prostaglandin $F_{2\alpha}$ were determined in gaseous and condensate phase, respectively.

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ENZYME INHIBITORS AS RECOGNITION ELEMENTS FOR MMP SENSORS

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Matrix metalloproteinases (MMPs) are a group of zinc containing endopeptidases involved in various pathologies. In particular, due to their role in wound healing as well as in cancer progression and metastasis development, there is a large interest towards new methodologies for the detection and monitoring of MMP-2 and MMP-9. In this work, the development of a capacitive biosensor for the detection of these MMPs is presented based on the idea that the strong affinities of MMP inhibitors could be used for a selective detection. Recently, the use of physisorbed MMP-inhibitors in a SPRI based detection scheme for MMPs showed the feasibility of this approach [1]. Covalent modifications of sensor surfaces with enzyme inhibitors for the detection of enzymes has been further reported by Whitesides and coworkers [2]. Bearing this in mind, we synthetisized a small library of probe molecules adapting the structure of reported inhibitors [3] to make it possible to bind them to a surface. The capability of these probes to bind the target molecules in solution was tested in silico and in a gelatin digestion assays, obtaining IC_{50} values in the low µM to low nM range.

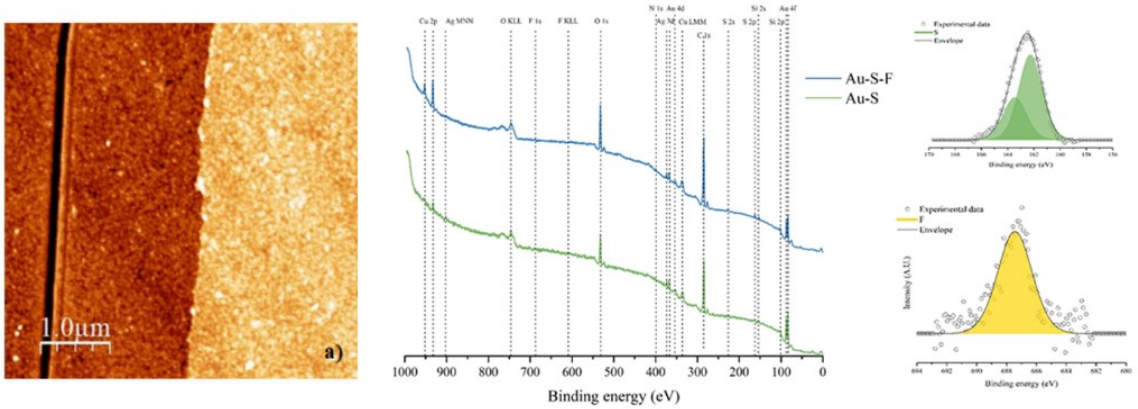

Figure 1. AFM micrograph of a functionalized gold electrode and XPS characterization of the surface proving the formation of a smooth homogenous monolayer as well as the functionalization with a probe molecule containing fluorine.

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A gold electrode was then modified by laying a self-assembled monolayer (SAM) bearing ω-ethylenglycol units to reduce non-specific adsorption. A homogenous and defect free surface was thus obtained (Figure 1), which was further activated with classical EDC/NHS conjugation chemistry and functionalized with the probe molecules, according to the method of Whitesides and coworkers [1].
Each surface functionalization step was monitored by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). These electrochemical techniques and AFM permitted to assess the roughness of the surface as well as to monitor the formation of SAM over time. EIS also allowed to monitor the degree of surface coverage as well as the surface activation and functionalization. The surface functionalization with inhibitor probes was further proven with XPS studies of a fluorine containing inhibitor analogue.
A PMMA flow cell was constructed and tested to house a three-electrode setup within a total volume of 100 µl. Preliminary tests against MMP-2 solutions showed the binding of the analyte to the surface. Future work will involve the measurement of MMPs in lysates of cancer cell lines and wound exudate.
Our intention is to provide an alternative to the two gold standard methodologies of MMP-detection, i.e. zymography and ELISA assays. We hypothesize that the first overestimates the amount of the active enzyme in a sample because the surfactant (SDS) dissociates natural TIMP inhibitors from MMPs, whereas the second cannot distinguish the active and inactive forms. Enzyme inhibitors only bind the active MMP forms, as shown in a study using sepharose beads conjugated with an inhibitor for affinity chromatography [4].
To our knowledge, this is the first example of an electrochemical detection of enzymes based on enzyme inhibitors, as examples with similar recognition schemes have so far been reported only with SPR and QCM methodologies.

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NEUROTOXIC COMPOUNDS DETECTION: A NOVEL ORIGAMI PAPER-BASED CHEMILUMINESCENT BIOSENSOR INTEGRATED WITH A SMARTPHONE DEVICE

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Global security threats have become a major worldwide concern and their early and sensitive detection represents a major challenge to current detection technologies. The routine monitoring of water, food and the environment for chemical and biological threat agents is often hampered by the fact that available techniques usually require clean samples and sophisticated equipment based on high performance liquid chromatography-tandem mass spectrometry and are thus unsuitable for real-time, cost-effective and on-field routine monitoring.
The possibility of implementing enzymatic assays with bio-chemiluminescence detection in smartphones has been very successful over the years, as well as the possibility of creating ad hoc analytical devices manufactured with an easy and economical 3D printing technology. Here, we report the development and optimization of a novel origami paper-based chemiluminescent (CL) biosensor for acetylcholinesterase activity detection and its implementation into portable analytical devices as proof-of-principle of low-cost point-of-care applications. This biosensor is based on the inhibition process of acetylcholinesterase (AChE) by molecules such as organophosphate pesticides, nerve gases and some drugs. The AchE activity is measured through a series of coupled enzymatic reactions leading to light emission. When acetylcholinesterase is inhibited, there is a decreased production of hydrogen peroxide, and consequently a reduction in light emission. In particular, three different enzymes, AChE, choline oxidase (ChOx) and Horse Radish Peroxidase (HRP), are adsorbed on a paper pad obtained by wax printing. The origami technique allows to add reagents in separate steps and trigger the reactions to occur sequentially. Due to its high affinity, tacrine, a competitive reversible inhibitor of AChE, was used as model analyte (concentration range 1 – 1000 μM). Hydrogen peroxide is produced based on the presence or absence of harmful substances acting on AChE. Signal acquisition was carried out by OnePlus 5 photocamera placing the 3D paper PAD cartridge inside a 3D-printed dark box and integrating CL signals for 30 sec with ISO800 (Fig. 1).

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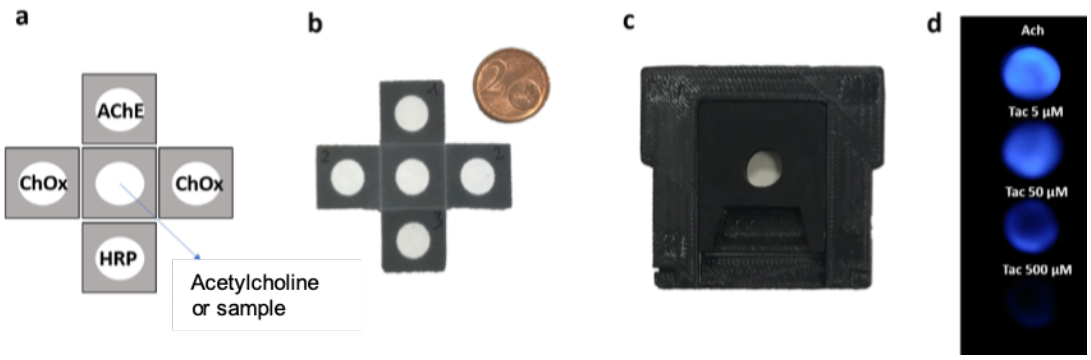

Figure 1. a) Schematic representation of origami paper-based chemiluminescent biosensor and b) Paper PAD obtained by the wax printing technique; c) 3D paper PAD cartridge d) CL signals acquired with OnePlus 5 smartphone.

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PREPARING AND TESTING IN POTENTIOMETRIC SETUPS SELF-STANDING CHIRAL MEMBRANES CONSISTING OF OPEN AND CYCLIC "INHERENTLY CHIRAL" THIOPHENE-BASED OLIGOMERS

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Recently, the "inherent chirality" strategy has been displaying more and more impressive enantiodiscrimination manifestations in chiral electroanalysis and electrochemistry experiments. [1,2] Such a strategy implies the use of chiral molecular selectors in which the same element, usually the whole main molecular backbone featuring a tailored torsion, is the source of both chirality and key functional properties; moreover, regioregular design can enable propagation and amplification of the peculiar features of a single unit in macro and supramolecular structures. After having successfully implemented inherent chirality in electrode surfaces [1] and, as an alternative, in ionic liquid media or related additives [2], we are now adding a new interesting tool in the growing palette of "inherently chiral" selectors, by introducing enantiopure self-standing membranes prepared by electrodeposition from enantiopure thiophene-based inherently chiral monomers followed by film detachment from the electrode support. Such membranes consist of a mixture of both open and cyclic oligomers, mainly dimers and trimers, in tunable ratio according to the electrodeposition conditions, as accounted for by high resolution LDI analysis. The membranes have been also characterized in terms of IR features and their morphology was carefully studied by SEM, TEM and AFM. As a preliminary investigation of functional properties, racemate membranes were implemented in a ISE-like setup, performing potentiometric tests with various achiral electrolytes at different concentrations; reliable transmembrane potential readings were obtained, quite consistent with those predicted considering the membrane and cell features [3].

The support of Regione Lombardia and Fondazione Cariplo (Project 2016-0923) is gratefully acknowledged.

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CHARACTERIZATION OF FUNCTIONALIZED GOLD GATES FOR EGOFET BIOSENSORS BY ATTENUATED TOTAL REFLECTION INFRARED SPECTROSCOPY

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Electrolyte-gated organic field effect transistor (EGOFET) biosensors are receiving increasing attention due to their ability to reach very low detection limits [1-3]. As a general concept, such unprecedented sensibility may be tailored by properly tuning the protocol for bioreceptor anchoring to the gate surface. Several approaches have been so far proposed for biosensor fabrication, namely physical adsorption, covalent strategies, and bioaffinity methods. As expected, physical methods are easy but poorly effective, whereas formation of covalent bonds allows for a good control and yield on bioreceptor immobilization without reducing their selective recognition properties. Considering the architecture of EGOFET biosensors involving a metal (generally gold) electrode as gate, the Au-S strong bond may be particularly advantageous to prepare biofunctionalized gate electrodes. Our research group recently developed an elegant strategy [4] to anchor a bioreceptor to gold surfaces starting with the formation of a self-assembled monolayer (SAM) from an ethanol solution of a mixture of 3-mercaptopropionic acid (3-MPA) and 11-mercaptoundecanoic acid (11-MUA) (10:1). The presence of reactive end groups (carboxylic moieties) allow for their successful activation via carbodiimide EDC cross-linking assisted by N-Hydroxysulfosuccinimide (NHSS). Afterwards, the modified gates are put in contact with bioreceptor buffered solution in order to achieve the integration of the biorecognition element to the gate surface. One (or more) blocking steps (such as with ethanolamine, EA) are then carried out to lower the probability of aspecific binding events, known to reduce biosensor analytical performance. It is then evident that the critical evaluation of the gate surface along all the steps of biofunctionalization protocol plays a central role in the understanding of biosensor working principle. Spectroscopic techniques may be particularly appealing, such as polarization modulation–infrared reflection adsorption spectroscopy (PM-IRRAS) may be applied to investigate SAMs on gold [5]. However, such an approach is not so widespread, and the use of more popular IR techniques could be appealing in this field. In this work, attenuated total reflectance infrared spectroscopy (ATR-IR) was successfully employed to probe functionalized gold surfaces at different steps of the modification protocol in order to gather

information about characteristic peaks ascribed to functionalities involved in the binding event and their eventual modification. As bioreceptor, biotin was selected for biofuctionalization of gold gates in EGOFET biosensors. A protocol for ATR-IR analysis was developed and used to characterize surfaces after each functionalization step. Spectra were acquired on different sample positions to evaluate process in-plane reproducibility. Spectroscopic results were essential to improve the biotin immobilization the protocol by a slight modification of the SAM growth step.

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MONITORING OF MICROBIAL GROWTH BY A POTENTIOMETRIC GRAPHENE-BASED SENSOR

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Chronic wounds affect about one million people just in Europe and generate high social and economic costs. The healing rate is quite low, in fact about 50% of ulcers are still open after 9 months. New technologies allowing a non-invasive, real-time assessment of wound conditions are actively sought from many research groups, without a real success so far. Wound bed pH has been correlated with the presence of microbial contamination, and a disposable pH sensor based on graphenic materials was developed in the framework of the SWAN-iCare project for the early detection of infections [1]. Notwithstanding the good accuracy of pH measurements in complex matrixes like plasma and wound exudate over many days, the early clinical data obtained with these sensors in the wound bed highlighted a remarkable positive shift from values measured with a reference glass electrode. In the present study, we report the results of experiments performed to investigate the possible role of bacteria in explaining such difference. It has to be considered that a biofilm community commonly develops in a chronic wound, which may affect the pH value due to the accumulation of metabolism by-products.
Potentiometric graphene-based sensors were tested in the presence of bacteria such as *P. aeruginosa*, *S. aureus* and *E. coli*, growing in the liquid culture media Luria Bertani. Four working electrodes (WE) coated with graphene oxide as the sensing material and one reference electrode (RE) Ag/AgCl were screen-printed on a thin and flexible polyethylene terephthalate (PET) substrate together with silver conducting tracks. Changes in the open circuit potential between WEs and RE were monitored in parallel to bacterial growth (expressed as the optical density, OD at 600 nm), and pH using a reference pH meter (glass electrode). For all the bacterial strains, sensor pH measurements did not match values from the reference glass electrode, but interestingly they correlated with the growth curves of *P. aeruginosa* and *E. coli*. In contrast, the stable sensor responses obtained with *S. aureus* were poorly correlated with glass electrode measurements and bacterial growth.
Further studies confirmed the presence of a bacterial biofilm on the sensor surface, which may account for a local surface pH change as a product of bacterial metabolism.

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CHARACTERIZATION OF A LABEL-FREE EGOFET-BASED PLATFORM FOR EARLY DETECTION OF HIV-1 P24 ANTIGEN

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The study and the understanding of the so called "wide-field" methods, used for new bio-sensing platforms, is extremely important in developing innovative device capable of analyte low-concentration detection [1]. In fact, fourth generation analytical tests aim at the early finding of those biomarkers that can induce an immune response from the infected host, and that are hardly detectable in the first stage of contagion [2]. For such a degenerative pathologies like AIDS caused by Human Immunodeficiency Virus (HIV) type 1 or 2, the prompt recognition of viral nucleic acid or HIV p24 antigen can reduce the diagnostic window within the acute phase of infection, giving the chance for antiretroviral therapies to be employed before the clinical symptom arrival. Different diagnostic tests are already available for surveillance, with two main approaches used depending on the test purposes: Enzyme-linked immunosorbent assay (ELISA), or rapid tests. They can be employed respectively if a bulky sample number has to be scanned and higher sensitivity is required, or if a fast screening is needed specially where the financial resources are limited [3]. The use of a point-of-care device that can detect single binding event between antigens and respective antibodies could satisfy the demand of a disposable but accurate analytical sensor. To this aim, an Electrolyte-gated Field Effect Transistor (EGOFET) was developed [4, 5], studying the bio-functionalization of a millimeter-sized gold gate on which the antibodies are immobilized. Through a self-assembly of mixed alkanethiols (3-mercaptopropionic acid, 3-MPA and 11-mercaptoundecanoic acid, 11-MUA), the anti-HIV-1p24 was bound to the gate surface, providing the transistor sensing component to be exposed to the analyte solution. The device performances were tested towards different HIV-1p24 concentrations, giving an electrical characterization of the system, related to the binding event.

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NOVEL APPROACH FOR DETECTION OF PEPTIDE HORMONES IN DOPING-CONTROL ANALYSIS

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Continuous improvements in the pharmaceutical industry have expanded the list of potential doping agents regulated and annually reviewed by the World Anti-Doping Agency (WADA) [1]. Protecting athletes' health during sport competitions and preventing the misuse of doping agents is of utmost importance. For this purpose, additional resources should be devoted to: increase the number of athletes tested, broadened the type of available assays and boost the frequency of out-of-competition controls [2].
Low molecular weight peptide hormones (<2000 Da), holding a well-defined structural characteristic, are a new frontier in antidoping research as these peptides have been included in the 2019 WADA List of Prohibited Substances and Methods [3, 4].
Among others, Gonadotropin-releasing hormones misuse in sports competitions has been reported. Gonadorelin, belongs to the GnRH drug class and is a neuro-decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly), available for medical use (e.g. for the treatment of hypogonadism, cancer etc.) stimulating the endogenous secretion of testosterone in the bloodstream via the hypothalamic-pituitary-gonadal (HPG) axis, eventually with impact on the athlete's biological passport [5]. Currently, there is a lack of miniaturized analytical methods to detect Gonadorelin in antidoping protocols. Therefore, we evaluated the possibility to develop a new, quick, efficient and sensitive assay based on biomimetic receptors, with the aim of determining Gonadorelin content in biological fluids (e.g. urine/plasma).
In this framework we report affinity interaction studies by surface plasmon resonance (SPR), between the analyte, Gonadorelin, and a specific nano-molecularly imprinted polymer (nano-MIP), successfully achieved for other peptides [6], for application in a competitive diagnostic assay format in microwelled plates with colorimetric detection.

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INNOVATIVE ELECTROCHEMICAL SENSOR FOR SINGLE-CULTIVAR CLASSIFICATION OF ITALIAN EXTRA-VIRGIN OLIVE OILS

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Extra virgin olive oils (EVOOs) are a key element of the "Mediterranean diet". Due to the huge market involved in south Europe, frauds concerning olive oil quality and origin are increasing in frequency. State of the art analytical techniques allow to identify such frauds but are often expensive and time consuming. The proposed sensor, a screen-printed electrode (SPE) modified with nanomaterials and generation IV ionic liquid [1,2], aims to perform quick screening analyses on EVOOs with minimum sample pre-treatment.
Monocultivar olive oil samples have been produced in our laboratory using olives hand-picked from trees of known cultivar. Before the analysis, olive oil was mixed with a solution containing lipase and incubated at 37°C in order to release the polyphenols contained [3]. After incubation, the mixture was dropped on the modified electrode and analyzed by means of cyclic voltammetry measures.
The results obtained in terms of potential and intensity of the anodic peak show a possible classification of the oils based on cultivar (Figure 1).
By comparing the results with those regarding the two previous harvesting seasons (2016 and 2017) a constant distribution of the different cultivar was observed.
The proposed electrochemical platform can be easily employed to construct a reliable, portable and easy to use instrumentation useful to perform screening analyses of EVOOs on the field.

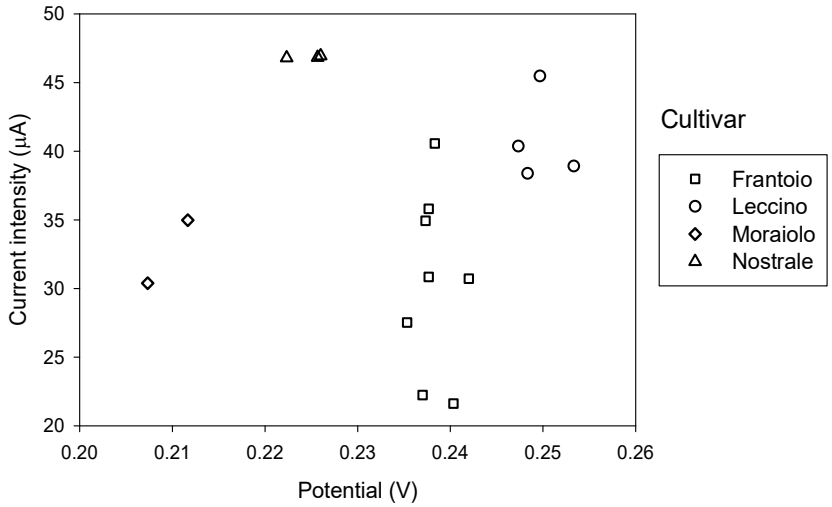

Figure 1. Classification of single-cultivar Italian olive oils harvested in 2017 using the proposed platform

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THE EFFECT OF THE ARTIFICIAL SALIVA COMPOSITION ON BRASS CORROSION

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Human saliva composition is complex and strongly variable from individual to individual. For this reason human saliva is usually not used for *in vivo* and *in vitro* studies, also because of its instability outside the oral cavity [1]. Several artificial saliva formulations have been proposed for electrochemical studies of alloys used in orthodontics. One of the most common solutions is the Tani-Zucchi formulation, which for example, was exploited to investigate the corrosion behavior of stainless steel used for braces production [2] and the surface modifications induced in historical brass wind instruments when they are played by musicians [3]. Tani-Zucchi saliva consists of a solution of inorganic salts (KSCN, KCl, NaH_2PO_4, $NaHCO_3$) with urea and α-amylase [4]. Following the contact with this solution, the surface of brasses was found to be strongly modified and the formation of a thick protective film mainly constituted by CuSCN and Zn-phosphate was revealed by X-ray photoelectron spectroscopy [3].
In this work, the electrochemical results obtained with the Tani-Zucchi solution on a CuZn37 brass sample are compared with those obtained with different saliva formulations such as Darvell, [5] Carter – Brugirard [6] and SALMO [7]. The electrochemical results will be combined with data from XPS surface analysis.
All these formulations [5-7] are more concentrated in the chloride, phosphate and urea concentration than the Tani-Zucchi one; among them, the Darvell formulation differs from the others also for the presence of organic compounds (uric acid, lactic acid and sodium citrate).

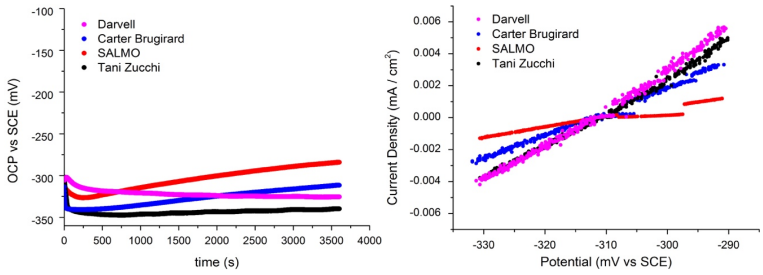

Figure 1. Left: Open Circuit Potential (OCP) vs contact time for the CuZn37 samples with the different artificial saliva solutions; right: current density vs potential by linear polarization experiments.

The electrochemical behavior of the brass samples with the different solutions was studied by means of open circuit potential measurements (OCP), polarization resistance measurements (R_p) by linear polarization experiments and potentiodynamic polarizations.

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The OCP was measured (Fig. 1) during 1h of contact in the different artificial saliva solutions open to air. The initial open circuit potential (E_0) was found to be -306 (6) mV for the brass in contact with all the solutions. For the brass in contact with the Darvell solution, the OCP vs time curves exhibited the same trend observed with the Tani-Zucchi formulation: the potential diminished upon contact time to more negative values, reaching a plateau after 1000s; the OCP measured after 1h was E_{1h} = -327(1) mV. This trend suggests an initial dissolution of CuZn37 in contact with the artificial saliva, then the formation of a surface film that could protect the brass from further corrosion. On the contrary, the OCP measured for brass in contact with SALMO and Carter-Brugirard formulations did not reach the plateau after 1h, and E_{1h} was more positive than E_0.
The polarization resistances Rp determined for Tani Zucchi, Darvell and Carter-Brugirard solution are comparable (about 6 (1) $k\Omega/cm^2$) while it was 16 (2) $k\Omega/cm^2$ for the SALMO solution. Rp values are inversely proportional to corrosion rate, thus the SALMO solution showed a three times lower corrosion rate compared to the other ones.
The interpretation of the electrochemical results can be performed exploiting X-ray photoelectron spectroscopy that allows the identification of the products formed at the brass surface upon contact with the solutions. Preliminary results showed that, for example, on the surface of CuZn37 in contact with the Darvell artificial saliva, nitrogen and sulfur were present but no P signals were detected: P signal was instead clearly revealed on the surface of CuZn37 samples in contact with Tani Zucchi saliva (Fig. 2).

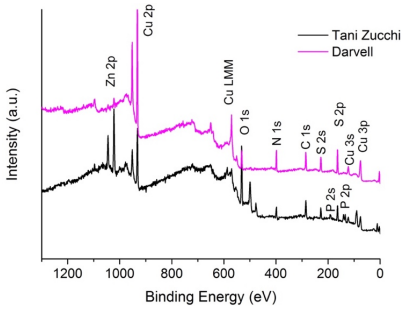

Figure 2. Survey spectra of CuZn37 after contact with artificial saliva solutions

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COMPARISON OF NIR AND FTIR-ATR SPECTROSCOPY IN THE IDENTIFICATION OF MARINE LITTER PLASTICS

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The presence of waste on the seabed is an emerging issue already highlighted by many studies, especially for the synthetic polymer fraction, commonly defined as plastics, which represents its most widespread component [1, 2]. It can negatively affect both the health of benthic and marine ecosystems as well as anthropic activities of economic interest such as fishery [3]. These negative interactions, however, could be changed into an interesting chance through collaborations and contributions from the fishing sectors, aimed to remove marine litter from marine ecosystems [4].
However, the recovery of waste from the sea is only the first step in solving this issue. Further challenges for the Marine Litter management rise from the absence or scarcity of environmental regulation in many European states, and from recent efforts toward a sustainable solution regarding the disposal of this waste. All solutions should comply with the provisions of the EU Directive 2008/98/EC, where a well-defined hierarchy is envisaged by the integrated management of waste, with a decreasing level of preference to apply from the prevention/reduction of waste production, to the reuse, recycling (recovery of materials) and energy recovery until, finally, to the landfill disposal.
The latter steps in the hierarchical management of Marine Litter require sound technological and scientific efforts for the classification and estimation of plastic wastes, such as waste category and chemical composition. These practices need to be focused toward a more sustainable and less environmentally impacting recovery of plastic marine waste, avoiding actions of an exclusive landfill disposal.
The commercial availability of instruments based on Near Infrared spectroscopy and customized for application in industrial processes and stock material control, allows also a rapid identification of plastic polymeric composition, representing an interesting chance for a more performing and implementable plastic recovery from Marine Litter. Likewise the recycling of plastics from urban waste, also polymers from ML must be separated and selected for a proper following reuse as raw material. For example, to produce fuel from plastic waste by pyrolysis, it is necessary to use polyolefins with low contaminations by other thermoplastic polymers [5, 6]. Among various spectroscopic instrumentations bearing technologies with an high level of miniaturization, those applying micro-electro-mechanical system (MEMS) seem more appealing, thanks to an electro-mechanical configurable chip changing the configuration of its pixels to act as a diffracting or reflecting element, allowing

the selection of different infrared light wavelengths dispersed by sample radiation towards an InGaAs detector [7, 8]. In the last decades, these portable instruments have assumed an important role in the control of products of commercial interest such as pharmaceuticals, food and virgin or recycled thermoplastic resins.

In this study, within the Interreg Italy-Croatia ML-REPAIR project, a handheld NIR spectrometer used in reflectance mode coupled with a micro-electro-mechanical system (MEMS) was employed to screen the plastic material recovered during the implementation of Fishing for Litter (FfL) activities, a consolidated practice of marine waste recovery during fishing and its following disposal in equipped port areas. This study had three main purposes: 1) to verify the applicability of this technique to the identification of plastic materials after environmental deterioration processes; 2) to compare the obtained analytical results with those attainable by established laboratory techniques such as FTIR-ATR; 3) to provide a preliminary estimation of the plastic waste composition recovered during FfL in two areas of the Adriatic Sea (northern coast in Italy and Dalmatian coast in Croatia). This last evaluation will allow to identify potential approaches to Marine Litter management, also based on the features of the investigated areas.

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NANOPARTICLE-ENHANCED SPR IMAGING AND PNA PROBES FOR ULTRASENSITIVE MICRORNA SENSING

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MicroRNAs (miRNAs) are short noncoding regulatory RNA molecules that are recognized as key players in various life processes. They also show promise as biomarkers for some critical diseases [1]. The analysis of miRNAs associated with cancer, freely circulating in the blood, highlights the importance of a simple and sensitive method for their detection and quantification, providing the basis of innovative cancer detection methods based on liquid biopsy [2]. However, several properties of miRNAs, including their small size and sequence similarities, combined with their low abundance in body fluids are crucial features to the development of the diagnostic assay in liquid biopsy [3].
Here, we report a sensitive and flexible method for targeting miRNA using Surface Plasmon Resonance imaging (SPRi) [4] and specifically designed Peptide Nucleic Acid (PNA) probes [5]. By combining the enhanced sensitivity of Nanoparticle Enhanced SPRi (NESPRi) with the use surface-oriented PNA probes [6], the detection of miRNAs in the low femtomolar range has been demonstrated. In particular, the immobilization of a PNA with horizontal orientation (T-shape), tethered to the gold surface through a lysine-modified backbone so that the probe had both termini accessible, allows for the modification of miRNA directly after the hybridization. Other possible orientations of PNA probes on the sensor surface and their consequences on accessibility of target miRNA by attaching at N-term or C-term, have been exploited in order to compare the selectivity of the assay.
An excellent SPRi signal amplification is then achieved by using the subsequent adsorption of properly functionalized gold nanoparticles (AuNPs) [7], which bind to the enzymatically added tails on target miRNA.
Moving from our proof-of-concept detection of synthetic miRNAs sequences, we have established and optimized a miRNA detection assay that is suitable for detecting miRNAs in biological extracts and in plasma samples from colon rectal cancer (CRC) patients as potential liquid biopsy candidates.
Moreover, we demonstrate an ultrasensitive sensing without complex and time-consuming procedures, making this assay a compelling alternative to traditional miRNAs detection methods requiring target amplification.

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GOLD NANOPARTICLES PRODUCTION FROM *ASPARAGUS OFFICINALIS* BY-PRODUCTS

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Research about nanomaterials is gaining growing interest in many fields of technology, thanks to the peculiar chemical, physical and mechanical characteristics that allow their use in several areas of applications [1]. Among the great number of nanomaterials, gold nanoparticles have attracted considerable attention for researchers all over the world due to their physical, chemical and optical properties that made them suitable for a wide number of application in many fields [2].
In recent years, researchers have considered the employment of green substrates for their synthesis. This approach is environment-friendly, and permits to work in a sustainability context. Many natural extracts have been evaluated as sources of compounds able to realize metal nanoparticles, such as algae extracts [3], which are rich in phenolic compounds and carbohydrates. Recent studies regarding the mechanism of metal nanoparticles formation demonstrated the involvement of hydroxyl groups of polyphenols or carbohydrate fractions, suggesting a possible role in redox reactions [4].
An important development, in agreement with the recent European directives aimed at reducing waste, and supporting the Circular Economy project, would be the reuse of agroindustrial by-products that today represent huge amounts of wasted materials. Those products are still rich of bioactive compounds, mainly constituted by polyphenols with antioxidant properties, and carbohydrates belonging to oligo and polysaccharides.
Keeping this in mind, this research is focused on the set up of a new method of synthesis of gold nanoparticles starting from natural extracts of *Asparagus officinalis* stems. They represent a non-edible part of the vegetable, and are normally discarded by the market and along the technological process of canned and under-oil food production.
Extracts of asparagus prepared with different solvents and at different concentration were tested as substrate for gold nanoparticles synthesis.
Analytical characterization of the extracts was carried out by spectrophotometric assay and chromatographic methods to evaluate the presence of polyphenolic compounds and carbohydrates. The results obtained by Folin Ciocolteu assay confirmed the presence of relevant amounts of phenolic compounds, responsible for a good oxidative stability, which

was measured by the Oxitest reactor. Separation of fractions according to their molecular weight was performed by steric exclusion chromatography, and revealed the presence of fractions corresponding to oligosaccharides and free sugars, that were also characterized by anionic exchange chromatography coupled to pulsed amperometric detector.
For the synthesis of gold nanoparticles, different experiments were performed on aqueous and alcoholic extracts at different concentrations until achievement of the best reaction conditions to attain narrow size distribution of the nanomaterials. Promising result were obtained as confirmed by UV-Vis spectroscopy, transmission electron microscopy (TEM), high resolution transmission electron microscopy (HRTEM), scanning transmission electron microscopy (STEM), electron energy loss spectroscopy (EELS) and energy-dispersive X-ray spectroscopy (EDX).
Analytical measures were also performed on the extracts after nanoparticles synthesis, to evaluate possible differences in the composition, with the aim of investigate the role of the different classes of compounds involved in the reaction.

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SYNTHESIS, CHARACTERIZATION AND DERMAL ABSORPTION THROUGH INTACT AND DAMAGED HUMAN SKIN OF CERIUM OXIDE NANOPARTICLES

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CeO_2 nanoparticles (NPs) are used in polishing products and absorbents, as promoters in wound healing and as organopesticides skin decontaminants. The European Agency for Safety and Health at Work (EU-OSHA) has listed CeO_2 NPs in the top five NPs worthy to be investigated as a priority [1]. While systemic bioaccumulation and organ toxicity has been described after inhalation, CeO_2 NPs transdermal permeation data are lacking. Since some applications of CeO_2 NPs presuppose the direct contact with the skin, their use has to be evaluated from a safety point of view, because a systemic uptake may pose toxicological side effects. The aim of the present study is to assess CeO_2 NPs dermal permeation using intact and damaged excised human skin, in order to evaluate their dermal exposure safety profile.
The CeO_2 NPs investigated in this study have been synthesized by a hydrothermal route using cerium ammonium nitrate and a synthetic sweat solution as precursors. The synthetic sweat used was the same as the one employed as donor fluid, consisting of 0.5% sodium chloride, 0.1% urea and 0.1% lactic acid in milliQ water, and pH adjusted to 4.5 using ammonia. The final dispersion was filtered over Amicon filters and the Ce concentration remaining in the supernatant solution was determined by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES). Ultrafiltration, Transmission Electron Microscopy (TEM) and Raman investigations were also performed for the NPs characterization.
Cutaneous absorption studies were performed using static diffusion cells following the Franz method [2]. Two different experiments were conducted using intact and damaged skin.
In intact skin permeation experiments, at time 0, the exposure chambers of 3 Franz diffusion cells were filled with 220 µL of the donor solution, corresponding to an amount of CeO_2 of 0.6 mg cm^{-2}, to ensure an infinite dose. The applied dose was the same of our previous studies on NPs permeation in order to better compare the results of the experiments [3,4].
After 24 h the donor phases, the receiving solutions and the skin pieces have been removed and stored in the freezer for the quantitative analyses.
Skin permeation experiments on damaged skin were conducted following the same procedure with the exception that skin was abraded by drawing the point of a 19-gauge hypodermic needle across the surface before the test.
For each experiment, one cell was added as blank. Each experiment has been repeated two times, in order to use the skin of four different donors.

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After the experiment, the skin pieces were separated into epidermis and dermis by heat shock, immerging in water at 60 °C for 1 and the skin layers were acid-digested in a closed microwave system (Multiwave PRO, Anton Paar).
ICP-MS Nexion 350X with an ESI autosampler, (Perkin Elmer, USA instrument) was used to determinate total Ce concentration in the receiver phases. The limit of detection of cerium was 0.001 μg L^{-1} for ICP-MS and the precision of the measurements as repeatability (RSD %) for the analysis was <5%.
Total Ce concentration in donor phases and in the solutions resulting from the mineralization of the skin samples were performed by ICP-OES using an Optima 8000 Spectrometer (PerkinElmer, U.S.A.), equipped with an S10 Autosampler. The precision of the measurements expressed as relative standard deviation (RSD %) for the analysis was always less than 5%.
The average amount of Ce into intact and damaged skin samples was 3.64 ± 0.15 and 7.07 ± 0.78 μg cm^{-2}, respectively (mean ± SD) (p=0.04) while in the receiving solution was 2.0 ± 0.4 and 3.3 ± 0.7 ng cm^{-2} (p=0.008). Ce content was higher in dermal layers of damaged skin compared to intact skin (2.93 ± 0.71 μg cm^{-2} and 0.39 ± 0.16 μg cm^{-2}, respectively p=0.004). Our data showed a very low dermal absorption and transdermal permeation of Ce, giving a first indication of Ce skin-uptake due to contact with nanosized CeO_2.
These results are comparable to that of our previous studies on the cutaneous absorption of other metal oxide nanoparticles [3,4]: this behavior is probably due to the very low ionization of these oxides in synthetic sweat resulting in a small concentration of free metal ions in the donor phase able to cross over the physiological barriers. The NPs capability of permeate the dermal layers is lower respect to the free ions and it depend on their physicochemical characteristics. These data represent an encouraging result for those CeO_2 NPs applications that involve the direct skin exposure.

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ELECTROSYNTHESIS OF ARBUTIN-LOADED COATINGS ON TITANIUM: DEVELOPMENT, CHARACTERIZATION AND BIOLOGICAL EVALUATIONS

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Oxidative stress is correlated to several bone pathological conditions, such as osteoporosis. Nevertheless, the redox state of bone cells has a significant impact on bone remodeling, even in physiological conditions [1]. Current research deals with protecting bone cells from oxidative damage, developing new strategies to control ROS levels, especially during prosthetic implantation. In this respect, arbutin could be exploited as antioxidant molecule on bone implants to protect the host tissue from oxidative damage. Arbutin is a glycosylated hydroquinone found in several plant species (*e.g.* blueberries, strawberries, wheat, pear trees) [2]. Recent studies described the effects of arbutin on bone cells, reporting a positive impact on osteoblasts' proliferation and differentiation [3] Other works highlighted arbutin's potential to attenuate oxidative stress in an *in vivo* model of Parkinson's disease [4], as well as to treat nerve damages [5].
The present study reports for the first time the protective effect of arbutin on osteoblast-like cells (Saos-2) and periosteum derived progenitor cells (PDPCs). Indeed, after exposure to arbutin, cells afforded an oxidative treatment with H_2O_2, reaching more than 80% of the viability over control cultures. Therefore, based on these results, an arbutin-loaded coating was prepared on titanium. The macromer poly (ethylene-glycol diacrylate) (PEGDA) was co-polymerized with acrylic acid (AA) by cyclic voltammetry, obtaining a PEGDA-AA coating. Electrochemical parameters were carefully optimized to reach a cytocompatible and homogeneous coating [6]. The latter was characterized by X-ray Photoelectron Spectroscopy (XPS) and loaded with arbutin during or after electrosynthesis. XPS analysis confirmed that arbutin's presence did not alter the growth of the coating. High Performance Liquid Chromatography was exploited to study arbutin amount loaded and released from PEGDA-AA coatings. The electropolymerized coatings allowed to tune arbutin loading, from 112±27μg/cm^2 to 5,1±0,4μg/cm^2 avoiding cytotoxic effects, as confirmed by viability assessment (MTT assay) and morphological evaluations (scanning electron and fluorescence microscopies). The reported data demonstrated the opportunity to synthesize an arbutin-loaded coating on titanium implants. Future investigations will shed light on the antioxidant and osteointegrative features of these arbutina-loaded PEGDA-AA coatings.

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DEVELOPMENT OF COMBINED ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY TECHNIQUES FOR Q.C. ON ELECTROPLATED METALLIC FILMS

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Corrosion resistance is a primary indicator of surface quality. Electrochemistry offers several techniques that can be used, alone or combined, for rapid determination of corrosion resistance and degradation rates. Electrochemical Impedance Spectroscopy (EIS) is a versatile procedure that can be used for fast evaluation of anti-corrosion performance of coatings: unlike other standard procedures is generally a non destructive method. EIS works applying an electrical sinusoidal potential of fixed frequency and measuring resulting alternate current. With this data electrical impedance Z (opposition that a circuit presents to a current when the sinusoidal wave is applied) of the sample can be calculated: measures are repeated at different frequencies and data are stored and properly plotted. In electrical theory impedance Z is a complex number that accounts for resistive and reactive components of the circuit: measuring impedance at different frequencies and analyzing the data we can postulate the structure of an equivalent circuit and extract corrosion resistance data. One of the objectives is to develop several methods (combining EIS and other electrochemical techniques such as open circuit potential OCP) to evaluate corrosion resistance of electroplated films used in fashion industry.

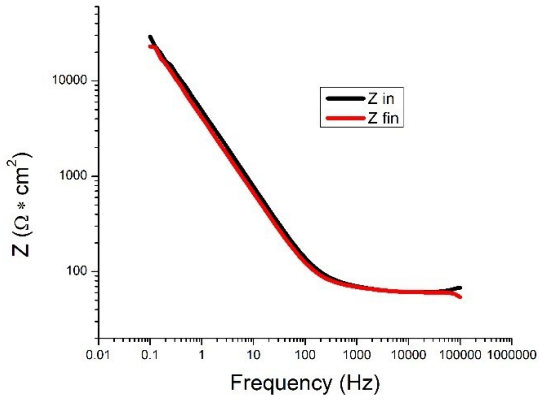

Figure 1. Comparison of EIS spectra of an electroplated metallic film immersed in electrolytic aqueous solution before (Z in) and (Z fin) after electrochemical induced stress at a cathodic potential

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DEVELOPMENT AND TESTING OF A NEW PROTOCOL TO PRECONCENTRATE NATURAL ORGANIC MATTER IN SURFACE WATER

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The term natural organic matter (NOM) is generally defined as all organic compounds (in dissolved and particulate forms), except synthetic molecules such as organic micropollutants, present in aquatic or terrestrial environments.
Hydrophobic acids form the major fraction of aquatic NOM, constituting more than half of the dissolved organic carbon (DOC) in water. These hydrophobic acids are often described as: (i) humic substances containing humic acids (HA), which are soluble in alkali, but insoluble in acid, (ii) fulvic acids (FA), which are soluble in both alkali and acid, and (iii) humins, which are insoluble in both alkali and acid [1]. Humic substances may constitute 95% of the total dissolved organic matter in aquatic system. These play an important role in aquatic chemistry and their presence can cause various environmental and health problems. For example, they bind with heavy metal ions facilitating their transporting in the water system [2]. In the last years, thallium contamination in water aroused interest due to its high toxicity and significant accumulation in human body [3]. Dissolved thallium can be found in two oxidation states, Tl (I) and Tl (III). Although Tl(I) is predicted to be more thermodynamically stable than Tl(III), photo-oxidation reactions and microbial activity, combined with the formation of stable hydroxo-complexes, contribute to the persistence of Tl(III) in surface waters.
In 2017, in *Department Environmental and Health, Water Quality and Health Unit, Italian National Institute of Health,* a multi sequential ultrasonic assisted extraction to determinate different thallium fraction on inner pipe surface was developed. Third step (extraction in oxidizing conditions) provides organic fraction release. ICP-MS analyses detect thallium contained in organic fraction. Moreover, the extent of Tl (III) complexation by natural organic matter (fulvic and humic acids) is unknown [4].
The aim of this study is to develop a procedure to concentrate all organic substances in surface water without fractionation that could interact with thallium. Because of the low concentration of dissolved organic matter (DOM) in surface water, the concentration procedure is needed.
In this work, the first step was a bibliographic research on preconcentration techniques applied to NOM and the analysis of different techniques to understanding the performance in terms of recovery.
Then, several techniques, in series, to concentrate most of DOM were tested.

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The pooled techniques are microfiltration, nanofiltration and reverse osmosis. Additionally, cation exchange extraction was applied to remove cation interference, before nanofiltration. The concentration procedure has been tested on a real case considering samples of surface water.

To this end, 25 litres surface water were sampled by pumping system and microfiltration (20, 1, 0.45 µm) was applied in field. The permeate solution was collected in glass tanks. In laboratory, total organic carbon was determined. Then, the permeate was passed through columns in series containing cation exchange resin, to remove Ca^{2+}, Mg^{2+}, Fe^{2+} and other interfering cations. Nanofiltration and reverse osmosis experiment were carried out on polyamide wrapped spiral membrane. After each filtration, DOC of permeate and concentrate solutions were measured by TOC analyser to assess recovery: concentrate solution was passed through membrane not less than three times.

The use of pooled techniques allows for concentrate most of dissolved organic matter and at the same time, it allows for decrease interfering species and it prevents the formation of membrane fouling.

This approach is the necessary basis to study organo-Tl complexes and to develop a method to characterize of the organic component that interact with thallium.

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FILM THICKNESS DETERMINATION USING A STANDARDLESS MONTE CARLO APPROACH THROUGH X-RAY FLUORESCENCE SPECTROSCOPY

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X-ray fluorescence is often employed in the measurement of the thickness of coatings [1]. Despite its widespread nature, the task is not straightforward because of the complex physics involved, which results in high dependence on matrix effects. Thickness quantification is accomplished using the Fundamental Parameters approach, adjusted with empirical measurements of standards with known composition and thickness. Unfortunately, there are no standards for any possible coating and coating architecture and, even relying on standards, the quantification of unknown samples requires the precise knowledge of the matrix nature. In this work, we describe a semiquantitative approach to coating thickness measurement based on the construction of calibration curves through simulated XRF spectra built with Monte Carlo simulations [2,3]. With this approach it is possible to simulate samples with virtually any kind of complex architecture and composition. Simulations have been performed with the freeware software XMI-MSIM. The sample used consisted in a copper substrate coated with palladium and gold with different thickness. We have assessed the accuracy of the methods by comparing the results with those obtained by XRF thickness determination with standards and FIB-SEM cross-sectioning. Then we evaluated which parameters are critical in this kind of indirect thickness measurement. The present study was funded by Regione Toscana within "Bando Servizi" projects of OBI and Eco-Tech Finish firms.

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GALVANIC ELECTRODEPOSITION AND SURFACE ANALYSIS

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The interest in scientific research within the metal finishing sector is growing. The demand for durable metals and adaptable manufacturing processes are increasing across a wide range of applications, from aerospace and automotive to machinery and jewelry. An essential step in the production line is the surface engineering of metals, as this determines the final appearance and functionality of a product. Therein electroplating is recognized as a mature technology allowing the low cost fabrication of defined surfaces with extensive property profile. Galvanic electrodeposition accounts today for almost 40% of the global market value share with North America and Western Europe leading the scenery. Although technological and processing advancements occurred in the past forty years, industrial firms are still struggling to provide solutions to corrosion protection as well as reduction of toxic wastes. Specifically, large-scale industrialization of electroplating techniques will continue to be limited by strict environmental regulations. Due to adverse ecological impacts, the adoption of plating processes involving toxic metals such as lead or cadmium is prohibited. Moreover, price volatility of the highly demanding electroplated materials gold, copper and nickel is expected to impact the market share for more than 60% by 2026.
In that respect, alloy plating offers better answers in terms of economic growth and environmental sustainability due to fine tuning composition, morphology and crystallinity [1]. Here, current trends on alloy electrodeposition research are reviewed highlighting open challenges and process innovations from an industrial perspective. The main categories of alloy compounds are presented and the most important properties for the manufacturing process discussed. Particular attention is devoted to advances in industrial quality control and viable solutions for the reduction of precious metal content in electroplated accessories as well as replacement of cyanide and nickel baths with non-toxic compounds.

The present study funded by Regione Toscana within POR Creo FESR 2014-2020, “EL4ALL”, “GADGET” and “Thin Fashion” projects.

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ANALYTICAL CHARACTERIZATION OF ULTRA-STABLE LASER-ABLATED METAL NANOPARTICLES AND THEIR ANTI-BIOFILM APPLICATIONS

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The application of metal and metal oxides in the form of nanophases, unveiling unprecedented physicochemical properties, has increasingly attracted the interest of materials scientists in different fields [1,2]. Among other features, these nanomaterials show a broad antimicrobial activity and can be advantageous to design bioactive coatings, with controlled metal ion release, exerting significant biological action and associated low toxicity for humans. In recent years, we have developed and characterized different nanoantimicrobial systems [3-5] offering a powerful alternative approach to fight bacterial resistance towards conventional antibiotics and disinfecting agents. In this study, bioactive Cu- and Ag- nanoparticles were produced as ultra-stable [6] nanocolloids by means of laser ablation synthesis in solution (LASiS) and they were used as water-insoluble nano-reservoirs of antimicrobial ions, when embedded in composite coatings. We exploited the key features of LASiS, a green and versatile route to nanoparticles, which does not require the use of any toxic reductant or stabilizer. Copper and silver colloids were synthesized by femto- or nano-second laser pulses in alcohol solutions. From an applicative point of view, the NPs stable in organic solvents are very interesting because they allow the preparation of blends with polymers and other hydrophobic molecules leading to water-insoluble composites. Nanomaterials were characterized by electron microscopies and molecular spectroscopies. Moreover, 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. Metal nanoparticles showed an unprecedented morphological stability towards aggregation over several months. On the basis of theoretical considerations and basic experiments, it is proposed that the stabilization of nanoparticles involves the formation of an organic coating generated by the interaction of isopropanol molecules with the pulsed, high-energy laser beam. This coating prevents, on the one hand, any chemical reaction on colloidal nanoparticles (e.g. oxidation); on the other hand, the presence of the organic shell with a nature akin to that of the organic solvent led to weaker Van Der Waals interactions between approaching nanoparticles enabling a larger stability than for naked metal nanoparticles. Transmission electron micrographs (Fig. 1) showed a thin organic shell, outlined as a low-contrast region surrounding the metal cores, resulting by IPA decomposition.

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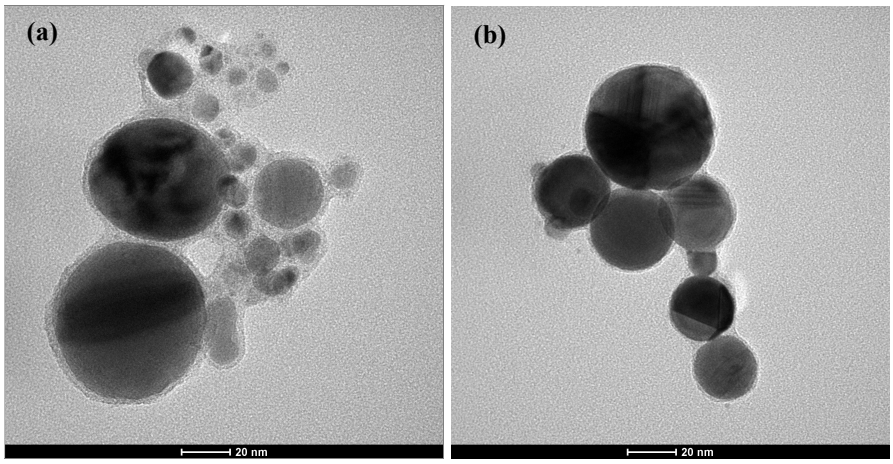

Figure 1. TEM micrographs at higher magnification of CuNPs (a) and AgNPs (b).

Bioactive nanocolloids were used as additives for the controlled modification of different biodegradable polymeric matrices and the mixed materials were used as precursors for bioactive and biodegradable multifunctional coatings. Antibacterial ion release kinetics from modified surfaces was monitored by means of electro-thermal atomic absorption spectroscopy, showing a tunable and long-term release of bioactive species over time. The risk of entire nanoparticle release was ruled out by electron microscopy investigations of the contact solutions. They were examined in different cases of study, including the development of active food packaging and bacteriostatic coatings for the automotive industry.

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SYSTEMATIC COLORIMETRIC MEASUREMENTS: FUNDAMENTAL QUALITY CONTROL TEST FOR FASHION ELECTROPLANTING INDUSTRIES

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Color measurement is one of the most important step in quality control at the end of the assembly line and in the research and development process for a variety of industrial applications printings, textiles, automotive and electroplating. Especially for electroplating, color inspection is a primary indicator of surface quality. Alteration of lightness, hue, and saturation are usually associated to surface defects or changes in galvanic baths composition and deposition efficiency. Though color measurements are easily accessible different instrumental settings and sample geometries might lead to uneven data. Variations in surface texture, angle of observation, deposition cycles and measurement protocols could drastically affect the reproducibility of analysis, thus resulting in communication problems between producer and customer. Specifically, in the fashion industry, the tiny dimensions and irregular patterns of accessories require the use of colorimeters with very small apertures and/or advanced analytical methods. The purpose of this study, focused on the fashion applications, is to take an overview of conditions and techniques of color evaluation, effectively implemented from companies, and to find out the most accurate, and at the same time economically sustainable one [1]. To reach this goal all the measurements are taken by keeping in mind the CIE's recommendations about standard observers, illuminants and operative conditions for reflective samples. Samples of variable colors were prepared on Ag substrates by electrodeposition of electroactive paints, known as cataphoresis, from commercial organic resins. Implications of variables such as sampled area, texture, and surface finish on colorimetric assessment are discussed. Laboratory outcomes were used to evaluate color accuracy and reproducibility of electrodeposited samples with different metal finishes among galvanic industries located in the Tuscan area. We anticipate our findings might be helpful to researchers and professionals working in fashion manufacturing and metal coating companies.

The present study funded by Regione Toscana within POR Creo FESR 2014-2020 and "Bandi Servizi": "GALVATRON" and "EL.PLA" projects respectively, in collaboration with ECO-TECH FINISH srl factory.

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MOLYBDENUM DISULFIDE THIN FILM ON SILVER SINGLE CRYSTAL BY E-ALD

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Thin films of semiconductors materials are taking great interest for their application in electronics and opto-electronics. To obtain high performance with these compounds it's requested a high controlled nanostructured surfaces ultra-thin films. One of these materials of interest in the fields of the material chemists, physicists and engineers is molybdenum disulfide (MoS_2). MoS_2 is a transition metal dichalcogenides (TMDC), the monolayers of this compound are 2-dimensional (2D) material, as graphene [1]. Each layer of MX_2 is a trilayer, made up of a sandwich of two chalcogen layers on either side of a transition metal center layer. The available method for bottom-up synthesis of large-area and uniform layers is chemical vapour deposition (CVD), although precise control of the number of layers over a large area has not yet been achieved. In this work, we present electrochemical measures for the development of an alternative bottom-up growth technique of MoS_2, the Electrochemical Atomic Layer Deposition (E-ALD). The E-ALD technique exploiting Underpotential Deposition (UPD), a type of Surface Limited Reactions (SLR), it enables the deposition of highly ordered ultra-thin films from diluted aqueous solutions and at room temperature and pressure. In the UPD an atomic layer of a first element is deposited on a second, at a potential before (below) that needs to deposit the first element on itself, so that the resulting deposit is generally limited to one layer atomic. 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.The E-ALD was used to grow molybdenum disulfide MoS_2 on crystalline Ag[111] electrode. UPD anodic electrodeposition of S^{2-} from ammoniacal buffer on crystalline Ag[111] electrode are well-known [2]. The research move to discover the optimal conditions to deposit Mo on Ag/S from a solution of MoO_4^{2-} in ammoniacal buffer.

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A PROPOSAL TO EXPLORE THE INTERPLAY AMONG SPATIAL AND SPECTRAL FEATURES IN HYPERSPECTRAL IMAGES

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We propose a methodological framework to extract characteristic images based on their spatial features in hyperspectral imaging data, while establishing a link to the spectral wavelengths where this spatial information is highlighted.
The approach relies on the 2D Wavelet Transform (by using the stationary wavelet transform implementation, 2D-SWT) capability of capturing distinct spatial features in disjoint subspaces (different sub-images at each spectral channel) and of multivariate data analysis tools to exploit this information. The data flow of the proposed methodology is illustrated on Figure 1: i) 2D-SWT is applied to the hyperspectral data cube, decomposing each image (without unfolding) corresponding to a single channel with a selected wavelet filter up to the maximum decomposition level (fig. 1.2); ii) the most distinctive features are determined by computing descriptors (such as contrast or homogeneity) on the grey-level co-occurrence matrices gathered by each sub-image obtained by 2D-SWT (per spectral channel) (fig. 1.3); iii) the descriptors are rearranged in a two dimensional data matrix, with the descriptors as rows and the spectral wavelengths as columns (fig. 1.4) and finally iv) this matrix can be investigated by different multivariate analysis tools to analyse the characteristic spatial features and their variability along the spectral channels, taking into account the correlation structure among spectral wavelengths.
In particular, it will be possible to depict the wavelet sub-images that carry the most relevant and distinctive information and at which spectral channels. This information can be further elaborated, e.g. to resolve pure spectral profile in multivariate curve resolution context.

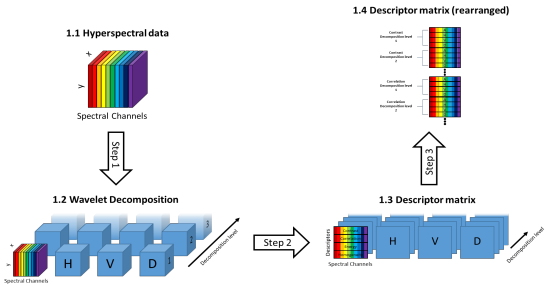

Figure 1. Illustration of descriptor matrix framework

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NMR-BASED METABOLOMIC FOR THE EVALUATION OF RED BEETROOT JUICE EFFECT ON ATHLETES DURING EXERCISE

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The growing interest concerning the strict relationship between diet and human organism is specifically connected to how alimentation affects people's health, activities and lifestyle. Food components, together with the specific need for certain nutrients, alter the cellular processes providing, simultaneously, outputs that derive from various and interconnected metabolic pathways. It has been proved that both external and intrinsic physiological factors influence the metabolic composition of biological fluids and that can be observed either with the appearance of unusual metabolites or with an increase or decrease in expected metabolites, thus leading to the identification of assumption's biomarkers as well as to the quantification of the direct diet effects on human metabolism. One of the most employed tools is the metabolomic analysis of urine. Urinary excretion is indeed the main way to eliminate all water-soluble waste products of metabolism such as products of the degradation of both exogenous substances and endogenous metabolites [1]. NMR-based metabolomic provide a valid tool to understand the multiparametric response of a living system to external stimuli, which can be related to foods, to a genetic mutation or to a drug's intake. The choice to investigate metabolome is due to a faster response and a real representation of the system at the exact time of analysis.
The aim of the present work consists on the evaluation of the effects that the assumption of red beetroot (*Beta vulgaris* L.) juice could have on athletes (cyclists).
Beetroots is a rich source of ascorbic acid, carotenoids, phenolic acids, flavonoids and also contain betalains, a group of bioactive pigments, categorised as betacyanin (pigments that are red-violet in colour) or betaxanthin (pigments that are yellow-orange in colour) with antioxidant and anti-inflammatory capabilities. They are characterized by high inorganic nitrate content on which are attributed effects on the vascular system: first via the entero-salivary cycle then via the stomach, NO_3^- is converted into NO, which acts as a vasodilator, consequently reducing blood pressure and thus preventing the onset of cardiovascular disease [2]. Furthermore, there are evidences that NO_3^- assumption increases tolerance to physical exercise. NO production is associated to NO_3^- intake. NO may inhibit oxidative ATP flux by competing with O_2 for the O_2-binding site at cytochrome-*c* oxidase (COX) in the

electron transport chain. COX inhibition of fibers nearest a capillary might allow O_2 to diffuse to fibers further from the capillary, thereby increasing "global" oxidative ATP production across a muscle. Improvements in muscle blood flow and a greater distribution of blood flow to type II muscle fibers with might also have contributed to the improved exercise performance [3]. Nevertheless, the observed effects are probably due more to the combined effects of other biochemical components contained in the beetroot juice, than the ones derived from the nitrate-nitrite-NO cycle only.

In this study the qualitative and quantitative characterization of the metabolic profile of the hydroalcoholic extract of red beetroot juice was carried out by ^{1}H and ^{13}C NMR spectroscopy. In greater detail, mono- and bi-dimensional experiments were acquired in order to univocally assign the NMR resonances originated by the water-soluble metabolites. The same approach was applied for the characterization of the metabolic urinary profile of the cyclists that assumed beetroot juice as well as placebo.

In a randomized crossover design, fifteen professional male cyclists completed a 2-days training session in which they consumed beetroot juice (BJ) or placebo (P) -which consists of a 10% diluted red beetroot juice with 10g/L sucrose correction. The team was divided into two groups in order to alternate the intake of red beetroot juice and placebo. Urine samples were collected at three different times, for both groups, for each day of the training session: after breakfast, during training and at the end of training.

Comparing the spectra of athletes' urine we observed that hypoxanthine resonances increase in intensity in the spectra at the end of training. These variations are the same for both BJ and P groups, which are probably linked to the metabolic pathways activated during the exercise, such as oxidation pathways involving hypoxanthine [4].

BJ group spectra, in addition to the quantitative variations, contains resonances that are completely absent in the P group spectra. Therefore, these signals (in particular the ones belonging to 2-carboxy-4-methylpiridine) can be ascribed to metabolites deriving from the intake of beetroot juice, making them as assumption biomarkers.

Moreover, hypoxanthine concentration increases during the exercise, as mentioned above, but this increment is higher in the urine from the P group compared to the BJ group.

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QUALITY BY DESIGN COMPLIANT STRATEGY FOR THE OPTIMIZATION OF THE EXTRACTION OF PHENOLIC COMPOUNDS FROM *VACCINIUM MYRTILLUS* BERRIES

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Bilberry (*Vaccinium myrtillus* L.) can be considered a functional food, as it has been recognized to provide several health-protecting attributes. The wide spectrum of its therapeutic effects has been suggested to be related to the high concentrations and wide variety of phenolic compounds, especially anthocyanins [1]. The development of effective extraction procedures for these compounds can be difficult due to their structural diversity and to their potent antioxidant activity, leading to a rapid reaction with other constituents in the matrix [2]. Mechanical Solvent Extraction (MSE) is an established and widely used procedure for the extraction of bioactive compounds from natural materials. Recently, Microwave-Assisted Extraction (MAE) and Ultrasonic-Assisted Extraction (UAE) have been considered as potential alternatives to the conventional MSE technique. Since many and significantly interacting factors are involved in the development of any extraction procedure, a multivariate strategy has been devised in order to carry out its optimization in a rational and scientific way.

In this study, a Quality by Design (QbD) approach [3] was applied in order to systematically compare the performances of MSE, MAE and UAE for maximizing the extraction efficiency of polyphenolic compounds from bilberry. QbD is a risk management-oriented methodology, which has been progressively integrated with analytical method development. To the best of our knowledge, this approach has not yet been reported for the development of extraction procedures. The QbD approach successfully allows the quality to be built into the process from the early stages of its development, returning a deep understanding of the whole procedure [4]. In the first step, the screening phase, the three techniques were investigated through screening matrices, selecting extraction time, kind of solvent, percentage of organic phase and sample/extractant ratio as critical method parameters (CMPs). In the case of MAE, temperature was also taken into account. The critical method attributes (CMAs) were chosen as the total phenolic content (TPC), the total monomeric anthocyanin (TMA), the antioxidant activity evaluated by 2,2-diphenyl-1-picrylhydrazyl assay (DPPH), the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay (ABTS), and Ferric Reducing Antioxidant Power Assay (FRAP). Graphic analysis of the effects made it possible to effectively compare the different extraction techniques and to select UAE for further optimization. The best performances obtained by UAE could be due to the ultrasound waves increasing mixing and

micro-mixing, promoting solvent penetration into the sample matrix and increasing the mass transfer rate of the antioxidant compounds into the extraction solvent. Through the same procedure, methanol was selected as solvent and a new experimental domain for the other CMPs was set on the basis of the screening results. A Response Surface Study was carried out and a Box-Behnken design was employed to find the coefficients of the quadratic models relating the CMPs to the CMAs. In this phase, further detailed information on the extraction process was obtained by means of HPLC-MS/MS analysis of the extracts, in order to evaluate the effect of the CMPs on the extraction of the most abundant polyphenolic compounds. A threshold value was set for each CMA and the sweet spot plots were drawn, highlighting the multidimensional zone where the requirements for all the CMAs were satisfied according to their predicted values. Hence, the method operable design region (MODR) was calculated by Monte-Carlo simulations [4], propagating the predictive error by using the model equations to the CMAs and computing the probability to reach the desired objectives. The threshold for the risk of failure was set to 10% and this risk was graphically plotted in the probability surfaces. Finally, the MODR was validated by verification points and an optimum working point was selected. This study pointed out how the great potential of a simple and fast extraction technique such as UAE can be effectively strengthened towards the production of phenolic natural extracts, through the implementation of a QbD approach.

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CHARACTERIZATION AND DISCRIMINATION OF TYPICAL ITALIAN PECORINO CHEESES BY MEANS OF HS-SPME/GC-MS AROMA PROFILING COMBINED WITH CHEMOMETRICS

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Pecorino cheese is an Italian diary product obtained from raw or thermized ewes whole milk. Milk is coagulated with different types of rennet and, after cutting, the curd can be cooked or not. After whey drainage and salting, cheese is ripened for a typical time in which its chemical composition and organoleptic properties evolve. Most varieties of Pecorino cheeses produced in Italy have a strong geographical identity, because the typical cheesemaking conditions adopted in specific territories, together with the origin of milk, affect their peculiar taste and flavour. In a number of cases, the relationship between the Pecorino cheese and the production area has been officially recognised by the attribution of specific certifications, including Protected Designation of Origin (PDO) [1].

In this work, we evaluated the potentiality of volatile profile in the characterization and discrimination of PDO Pecorino Romano (PR), PDO Pecorino Sardo (PS) and Pecorino di Farindola (PF) cheeses. PF, which has been included by Slow Food Foundation for Biodiversity in the list of traditional food to safeguard, is the only Italian cheese and perhaps in the world made with pig rennet, an unusual practice dating back to Roman times. More stable rennets, such as lamb and calf paste, are used instead in the production of PR and PS Pecorino cheeses, respectively. The kind of rennet is considered an important factor in the cheesemaking process and is expected to influence the evolution of cheese sensorial properties [2]. Concerning the geographical origin of Pecorino cheeses, PS is only made from milk produced in Sardinia, while PR is produced with milk coming from Sardinia, Lazio or Province of Grosseto (Tuscany). PF, by contrast, is produced in a restricted mountain area within the National Park of Gran Sasso and Monti della Laga located in the central Apennines (Abruzzo).

To attempt a discrimination of the above three typical Pecorino cheeses, representative samples belonging to each type were analysed by GC-MS combined with headspace solid-phase microextraction (HS-SPME). The HS-SPME/GC-MS method was preliminarily optimised using a design of experiments combined with surface response methodology (Figure 1). The HS-SPME/GC-MS data collected under the optimised conditions were finally handled by both supervised and unsupervised multivariate statistical approaches to classify the Pecorino samples.

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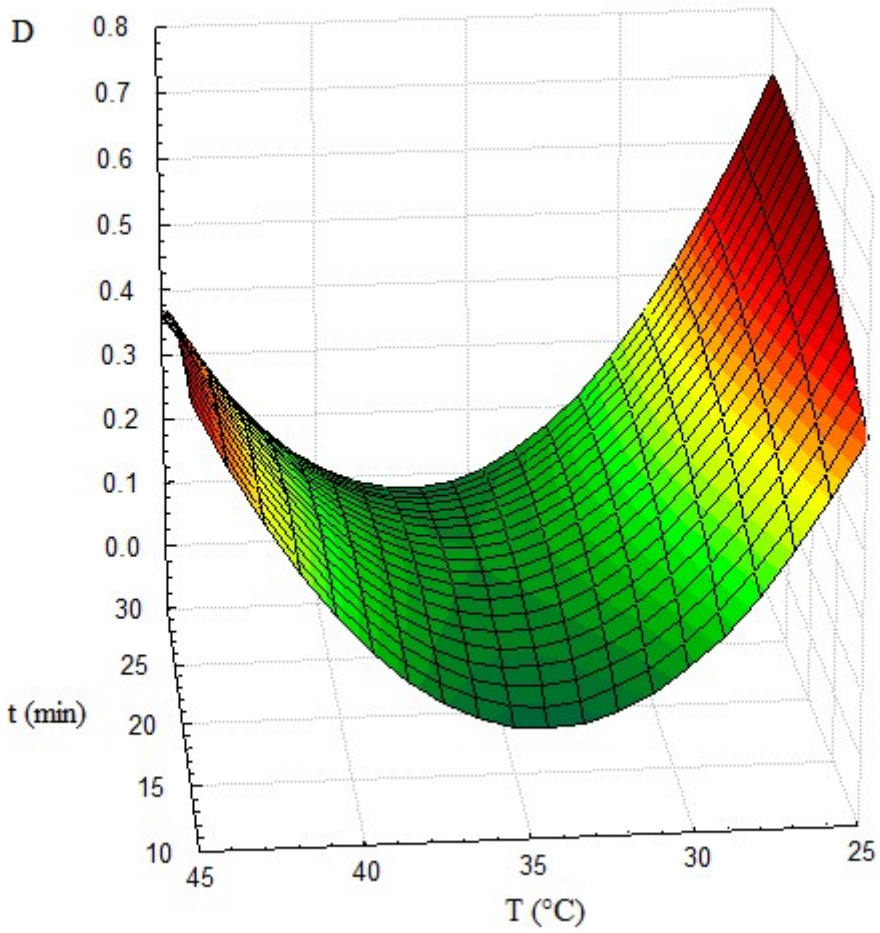

Figure 1. Response surface plot for global desiderability (D) related with number and intensity of chromatographic peaks as a function of sample temperature (T) and fiber exposure time (t) in SPME-HS extraction.

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GEOGRAPHICAL DISCRIMINATION OF LENTILS (*Lens culinaris* Medik.) BY ICP-OES MULTI-ELEMENTAL ANALYSIS AND CHEMOMETRICS

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Lentil (*Lens culinaris* Medik.) is an old domesticated grain legume cultivated worldwide and known since ancient times for its therapeutic and high nutritional value. Lentil crop exhibits high adaptability and, due to the short life-cycle, has spread even in low input semi-arid areas. In Italy, lentil growing is based on well adapted landraces which are the outcome of a long-time selection empirically performed by farmers [1]. It follows that the biodiversity of Italian lentil is closely related to tradition that makes some varieties cultivated in specific territories unique and valuable. However, most of lentil marketed in Italy is imported from Canada that is the major producer (48 % of global production in 2016) and exporter country in the world. Therefore, discrimination of the authentic Italian lentil varieties from non-European ones is an important aspect to safeguard consumers and traders from frauds based on mislabelling or marketing of blended products [2]. In the present work, multi-elemental composition of typical Italian and Canadian lentil samples was determined and geographical classification, using both discriminant and modeling pattern-recognition methods, was attempted. After microwave assisted digestion, 69 Italian lentil samples, produced in three relatively close areas located in the Apennine region of central Italy (Castelluccio di Norcia, Colfiorito and Santo Stefano di Sessanio), and 20 Canadian imported samples were analysed by means of inductively coupled plasma optical emission spectrometry (ICP-OES). The content of fifteen elements was evaluated to assess the usefulness of mineral composition in the geographical traceability of lentil seed samples. After preliminary exploration by means of principal component analysis (PCA), supervised classification methods were applied, i.e. linear discriminant analysis (LDA), partial least squares-discriminant analysis (PLS-DA) and soft independent model class analogy (SIMCA) [3]. The efficiency and reliability of the classification models were assessed on 27 external samples selected by duplex Kennard-Stone algorithm. Step-wise LDA based on the ten most discriminant variables (Ba, Ca, Cu, Fe, Mn, Mo, Ni, Mg, P, Zn) allowed to classify 100% of the samples of both calibration and prediction sets (figure 1). PLS-DA, with an optimum complexity of 13 latent variables, exhibited good discrimination ability both in calibration and prediction. The SIMCA class models presented high sensitivity (all the calibration and external samples were correctly accepted by the related classes) and good specificity since most of non-compliant samples were refused by each modelled class.

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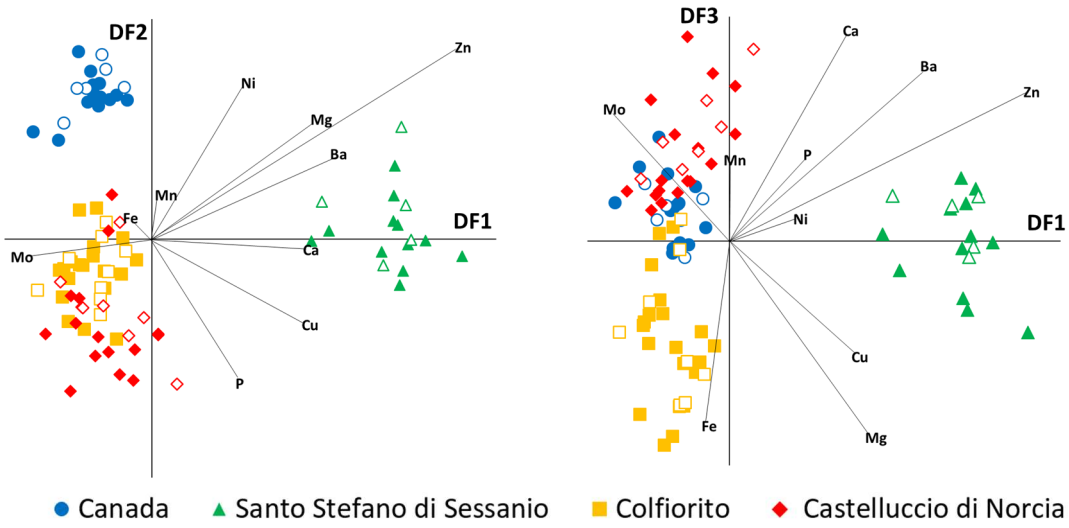

Figure 1. Lentil samples differentiated according to geographical origin by linear discriminant analysis. Full and open symbols represent calibration and external samples, respectively.

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RARE HEMOGLOBIN VARIANT IDENTIFICATION BY TG/CHEMOMETRIC SCREENING TEST

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Thermogravimetry coupled with chemometrics proved to be able in the β-thalassemia screening and in the differentiation of patients according to the clinical severity of the disease; in addition, the effectiveness of its prediction ability demonstrated to be not influenced by drug therapies such as aspirin, commonly used by thalassemia patients who undergo splenectomy to prevent thromboembolic event [1-4].
In this study, the new TG/Chemometric screening test is applied to solve a difficult case of congenital hemolytic anemia, caused by an unknown defect.
Termogravimetry was used to investigate a whole blood sample taken from a 6 years old girl, monitored at the Hematology Unit of the Bambino Gesù Hospital of Rome affected by chronic hemolytic anemia. Thermal profile recorded on patient blood sample was compared with those of healthy and thalassemia subjects and processed by the developed PLS-DA prediction model for thalassemia screening. The blood sample was identified as belonging to the class of thalassemia patients, identifying a chronic anemic state and suggesting the presence of hemoglobin defect.
The presence of a very rare hemoglobin variant Hb Bibba (alpha-2-136Pro (H19) -beta2) was confirmed by molecular characterization of α and β globin genes, carried out at the Microcythemia Center in Rome.
In conclusion, the TG/Chemometric approach allowed a rapid and cost effective identification of this case of congenital hemolytic anemia of difficult diagnosis, while the conventional screening test including the differential diagnosis of hereditary hemolytic anemia, the erythrocyte osmotic resistance, the electrophoretic analysis of erythrocyte membrane proteins and the study of the main erythrocyte enzymes, were not sufficient enough for diagnosis.
The study suggest the possibility of using the innovative TG/Chemometric screening test to detect rare hemoglobin variants and to expand its application to pediatric patients as it requires small sample volumes and is able to characterize patients subjected to transfusion.

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A NANOSPHERICAL DENDRIMERIC GALLATE ESTER FOR LONG TERM PRESERVATION OF ESSENTIAL OILS: AN INTEGRATED CHEMOMETRIC ASSISTED FT-IR STUDY

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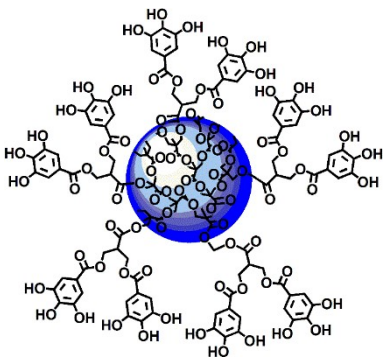
Essential oils (EOs) are hydrophobic concentrated liquids from plants made of volatile chemical compounds. EOs are very popular in the food, cosmetic and pharmaceutical industry as aromas, fragrances and alternative therapeutic devices [1, 2]. EOs are susceptible to degradation reactions, especially of oxidative type, triggered by temperature, light and oxygen availability. A loss of quality and alterations of sensory and pharmacological properties may occur, causing the production of smelly or even harmful compounds, responsible for allergic reactions and skin irritation [3-5]. For preventing and delaying EOs' spoilage, synthetic preservatives as 2,6-*bis*(1,1-dimetiletil)-4-metilphenol (BHT) or *t*-butil-4-hydrohyanisole (BHA) are commonly adopted; but, in addition to a limited efficiency due mainly to poor solubility in oils, they may cause health diseases [6]. Natural polyphenols as gallic acid (GA) are nowadays proposed as safer alternatives, but their efficiency is limited by their low compatibility with hydrophobic material again, or by the occurrence of probable side reactions with oils constituents. Recently, a hydrophobic and biodegradable GA-enriched dendrimer (GAD) (Fig. 1.a) characterised by a nanospherical morphology (Fig. 1.b) and endowed with a remarkable antioxidant activity was synthetized [7]. Further studies currently being completed, have shown that GAD, with respect to free GA, possesses also more efficient antibacterial properties against several antibiotics-resistant G+ strains, inhibits platelet aggregation and ROS accumulation thus representing an excellent alternative to conventional drugs to combat infections and thrombus formation [8]. In this study, based on integrated results obtained from the due investigations, GAD is advised also as an innovative and semi-synthetic preservative additive.

a)



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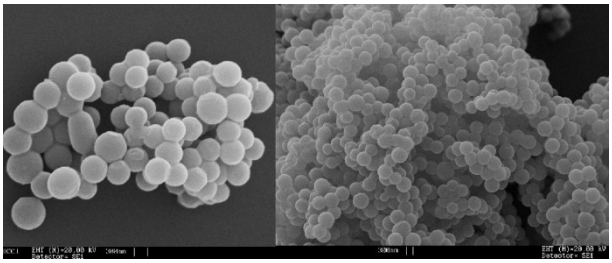

Figure 1. Intuitive representation of GA-enriched dendrimer (GAD) structure (a); SEM images of GAD spherical nanoparticles (b). Scale bars represent 300 nm.

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In this regard, GAD proved a much more efficient preservative power than free GA and, unlike GA, it never acts as a pro-oxidant. Besides classic oxidation indexes, the desired information was obtained by FT-IR spectroscopy assisted by multivariate analysis (MVA). For further confirmation of the so obtained results, interpretations of FT-IR data by considering the area of some selected informative bands and iodometric titrations to determine the hydro peroxide value (PV) were also performed [9].

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PERSULFATE ACTIVATION BY HYBRID MAGNETITE/HUMIC ACID NANOPARTICLES FOR THE PHOTODEGRADATION OF BISPHENOL A

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Advanced oxidation processes (AOPs) have been deeply studied since several decades due to their potentiality to yield the mineralization of organic pollutants, thanks to the generation of very reactive oxidant species (mainly hydroxyl radicals, •OH).
Fenton, photo-Fenton and Fenton-like processes, amongst AOPs, have been attracting wide attention to degrade recalcitrant organic pollutants in water [1]; in the "classic" Fenton process, the highly reactive species are generated by the reaction between Fe(II) ions and hydrogen peroxide. The reaction strongly depends on the pH, featuring its optimum at pH 3, needing therefore a subsequent neutralization step, with sludge formation and increase of the overall cost of the process. In the heterogeneous Fenton and photo-Fenton reactions H_2O_2 is activated by iron supported in a solid matrix with the advantages of allowing the catalyst recovery and reuse, avoiding the sludge formation and significantly simplifying the process [2]. In previous work we proposed the use of humic acid(-like) coated magnetite nanoparticles (HMNPs) for the heterogeneous photo-Fenton degradation of caffeine, obtaining promising results at circumneutral pH values [3].
Based on these premises we developed HMNPs by co-precipitation method under controlled anoxic conditions using different amounts of humic acid; from preliminary experiments we identified the best material in term of humic acid loading and we coded it Fe_3O_4/0.5HA.
Besides H_2O_2, it has been reported the possibility to activate persulfate by irradiated magnetite, generating radical species able to promote phenol degradation [4]. Therefore we decided to test the capability of Fe_3O_4/0.5HA to activate persulfate in order to run the heterogeneous photo Fenton-like degradation of bisphenol A (BPA), a compound widely used as raw material in the manufacturing of numerous chemical products, such as polycarbonate plastics and epoxy resins. In 2017 the European Chemicals Agency listed BPA as a substance of very high concern due to its properties as an endocrine disruptor and since it has been detected in treated drinking water, surface waters, effluents from wastewater treatment plants, landfill leachates, sediments, there is a great interest in optimizing its degradation.
In the present work we studied BPA photodegradation in the presence of Fe_3O_4/0.5HA and sodium persulfate, under UVB irradiation. We studied the effect of pH and persulfate concentration, we tried to identify the most important reactive species driving BPA degradation , we run experiments in real water samples (real effluent of water treatment

plant) and we checked the possibility to recover Fe_3O_4/0.5HA and re-use it in further water purification cycles.

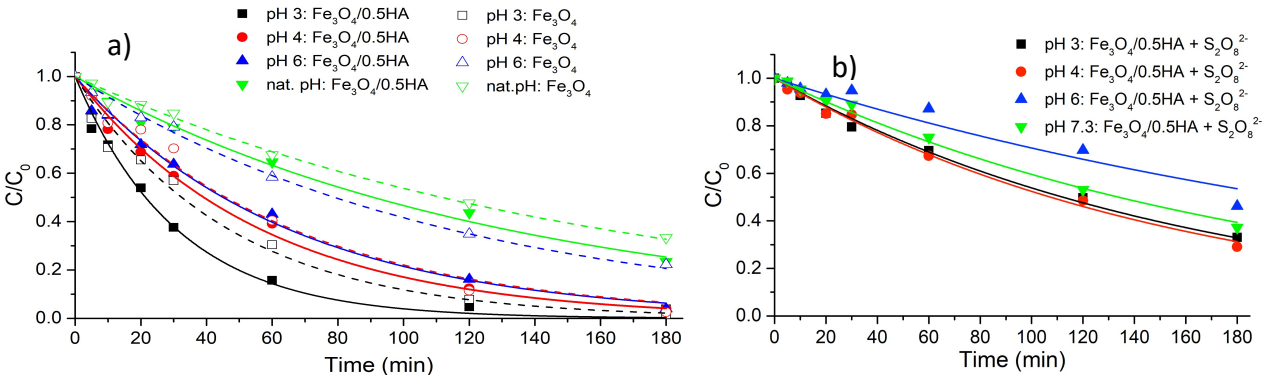

Figure 1. BPA degradation vs irradiation time in a) milliQ water and b) real water matrix.
$[BPA]_0$ = 20 μM; $[Fe_3O_4/0.5HA]_0$ = 100 mg/L; $[S_2O_8^{2-}]_0$ = 1 mM

As can be observed in Figure 1, the best results have been obtained in MilliQ water at pH =3, but BPA degradation is still possible also at neutral pH in a real water matrix, encouraging further study in this direction.

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DEVELOPMENT OF A NEW HOME-CARE DETERGENT: EXPERIMENTAL DESIGN IN THE OPTIMIZATION OF PERFORMANCES AND STUDY ON THE SAFETY OF USE THROUGH PHYSICO-CHEMICAL AND TOXICOLOGICAL DATA

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Manual dishwashing is still used by a great portion of the households. The formulation of a new dishwashing detergent was optimized with the aim of obtaining a product which could be effective in the field of detergency, but at the same time could be safely used without the need to wear gloves. The development process involved the chemical analysis of the market leaders in the home-care detergent field and therefore the identification of the main parameters to be satisfied: dry residue>10%, as an index of the amount of surfactants in the formulation, and viscosity>800 mPa·s. On the basis of these preliminary data, a formulation hypothesis was implemented and then further tuned by an experimental design strategy. The selected factors were the percentages of the surfactants sodium lauryl sulfate, cocamidopropyl betaine, disodium capryloyl glutamate and of potassium cocoate soap. The investigated responses consisted in foam formation, as an index of the emulsifying capacity and therefore of cleaning performance; viscosity, identified as an organoleptic characteristic appreciated by the consumers; variation of the degree of skin hydration when the product is applied, as an index of safety of use; pH, due to its role in both solution equilibria and product conservation. A Central Composite Design was employed for calculating the quadratic models relating the factors to the responses and contour plots were drawn. For all the responses a target value was fixed and sweet spot plots made it possible to identify the zone where all the responses were simultaneously optimized. An optimum point was identified and the corresponding formulation was prepared, verifying the agreement between the measured and predicted responses. The suitability of the new formulation was assessed through the execution of stability tests, pH monitoring and organoleptic properties evaluation. In order to ascertain the safety of use of the new product, both a theoretical study based on Margin of Safety (MOS) [1] and a measurement of the variation of skin hydration caused by the application of the developed formulation were carried out, confirming the good safety properties of the novel formulation.

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DEVELOPMENT OF NEW STRATEGIES FOR THE RESTORATION OF SOILS DESTINED FOR URBAN HORTICULTURE: ABATEMENT OF ORGANIC POLLUTANTS BY BIODEGRADATION

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Recently, municipal administrations have shown a growing interest in the restoration of urban and peripheral green areas using them to cultivate flowers, fruit and vegetables.
Urban horticulture presents various positive aspects since, in addition to favouring an environmentally friendly urban regeneration, it promotes sustainable cultivation and zero-kilometres products consumption, social aggregation and environmental education of citizens.
However, urban soils might be exposed to pollutants, such as recalcitrant organic substances (e.g. polycyclic aromatic hydrocarbons (PAHs)) and heavy metals, especially if they are close to roads and to vehicular traffic. For this reason, it is essential to assess if vegetable garden products intended for consumption are not subjected to contamination and are not dangerous for human health; besides, if the concentrations of pollutants in urban soil exceed legal limits it is necessary to develop soil purification strategies.
The aim of this research project is to develop new strategies for the recovery of contaminated soils, which should have a low environmental impact and a low cost. Phytoremediation could be the answer concerning heavy metals abatement, whereas biodegradation, via bioaugmentation of autochthonous microorganisms can be exploited for soils with a high organic pollutants content.
As regard biodegradation, particular attention was given to the autochthonous microbial population of the contaminated soil, because it is hopefully adapted to this extreme environment. Before proceeding with the in-situ treatment, the screening of the autochthonous fungi and the identification of the most suitable ones was required. The cultivable microflora was assessed by soil dilution plate and enrichment techniques. Identification of autochthonous strains was performed by means of a polyphasic approach that combines morphological and molecular methods.
Those microorganisms able to degrade organic pollutants, exploiting them as source of nourishment, were identified by means of a miniaturized screening test. Since bioavailability is a critical issue for the organic pollutants soil contamination, the capability of fungi to produce biosurfactants was also assessed. Microorganisms classified as potentially harmful for human and animals were excluded for safety reasons, while the best performing ones against the pollutants of interest were evaluated for the biological treatment of the soil.

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They were pre-cultivated in an appropriate ligninocellulosic substrate in order to carry the fungal mycelium in field. Before proceeding with the inoculation, and after a preliminary homogenization process of the soil of interest, the initial concentration of PAHs was measured.
In field bioaugmentation was performed on a central area (via Campana) in Turin, where high concentrations of some heavy metals and PAHs were detected. Four experimental theses were assessed: soil in its unaltered state, soil added with microorganisms, soil added with amendment, supplied by ACEA Pinerolese and soil added with microorganisms and amendment. In this way, it was possible to assess the efficiency of the process and the contribution given by the addition of a soil improver to the biodegradation.
Pollutants content was constantly monitored on the restored soil both by high-performance liquid chromatography with fluorescence detection and by gas chromatography- mass spectrometry. Besides ecotoxicological analysis were performed, coupling this information with the analytical results.

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USE OF BIOCHAR AS WATER PURIFICATION SYSTEM IN WATERS CONTAMINATED BY HEAVY METALS

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Biochar is a carbonaceous material made by pyrolysis or gasification processes from different types of vegetable biomass.
It is generally used in several agricultural and environmental applications as a soil improver.
Biochar can be also used as an adsorbent for organic and inorganic contaminants, especially for heavy metal removal. This absorbing capacity is due to its high pH (range), extensive surface area, anionic and cationic exchange capacity. These properties are achieved according to different pyrolysis temperatures applied to the processes. For instance, high temperatures during the pyrolysis induce a higher absorption of organic contaminants by the biochar itself. Biochar pollutants absorption capacity is excellent in contaminated soil treatment, but it could be also used in water treatment intended to human consumption. To achieve this goal, the study was focused on the evaluation of biochar absorption capacity starting from different vegetable essences (such as spruce, corn cob, sawdust, hazelnut shells and so on), producing it at different temperatures. Biochar is usually made at pyrolysis temperatures of 500 ° C. In this work, instead, all the samples were made at three different temperatures, such as 550 ° C, 800 ° C and 1100 ° C. Samples of biochar were undergone at two different treatments: the first one was the naturally release of occurring metals, the second one was the metal removal (Al, Cd, Cr, Cu, Mn, Ni, Pb and Zn) into a water solution at different pH. In the first case biochar samples were treated with aqueous solutions at three different pHs and left for 72 hours in stirring. These operations allowed us to evaluate how many metals could be released from the biochar itself. Subsequently, metal absorption capacity of biochar was tested in a weakly acidic and neutral environment. The results shown that biochar is a good and encouraging retainer of these metals, especially for aluminum, chromium, copper, lead and nickel.

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SUNLIGHT PHOTODEGRADATION OF CYTOTOXIC DRUGS. IDENTIFICATION OF PHOTODEGRADATION PRODUCTS BY UHPLC-MS/MS IN WATER SAMPLES

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The identification and quantitation of emerging contaminants of concern in water from both surface and underground sources is extensively covered in the literature [1]. In relation to this, the investigation of degradation products of emerging contaminants in water by employing different degradation techniques has been a focus research [2].
In this study, the photodegradation of four antitumoral drugs, irinotecan, topotecan, gemcitabine and mitomycin, has been investigated. The drugs are among the most widely used drugs in cytotoxic chemotherapy [3], used to treat colon cancer and small lung cancer like irinotecan, ovarian cancer, small lung cancer and cervical cancer like topotecan, cancer of the bladder, pancreas, ovary and breast, and non-small lung cancer like gemcitabine, and cancer of the breast, pancreas, stomach, anal, lung and liver like mitomycin.
The photodegradation study was carried out by exposing solutions of the drugs, prepared in MilliQ water, to simulated solar irradiation in a SOLARBOX®. The solarbox irradiation simulates degradation of the chemotherapeutic drugs in surface water.
Exactly 28 mL solutions (10 mg L^{-1}) of the drugs were placed in cylindrical quartz cuvettes and were subjected to the solarbox irradiation, which was carried out for up to 13 days. Sample aliquots of about 3 mL were withdrawn after irradiation to predefined time intervals. To monitor the progress of the photodegradation processes, the sample aliquots were subsequently analyzed by UV-vis spectrophotometer.

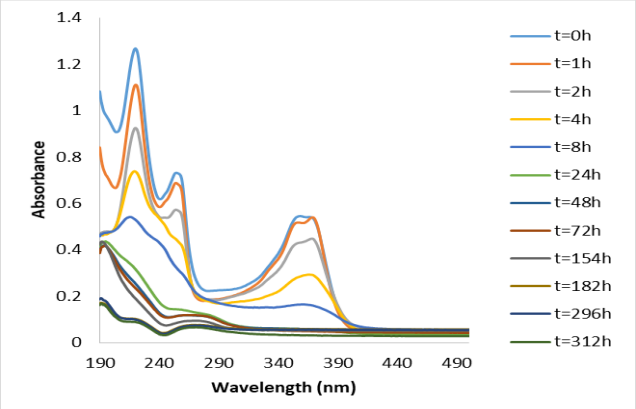

Figure 1. The photodegradation of irinotecan: UV light absorbance spectrum measured as a function of solar light irradiation time.

UV spectral measurements showed that about 90% of the initial amounts of mitomycin, irinotecan and topotecan were photodegraded after 1.0, 7.6 and 12.3 days, respectively. Gemcitabine was found to be more resistant with about 40% degradation in 13 days of irradiation. The UV spectral profile of irinotecan, for example, is shown in Figure 1.
Furthermore, the separation and identification of irinotecan photodegradation products was performed by UHPLC-MS/MS. First, a preliminary MS/MS characterization study of irinotecan in positive ion mode was performed and the quasi-molecular ion $[M+H]^+$ was detected with good signal intensity at *m/z* 587. Figure 2 shows the formation of photodegradation products of irinotecan (only profiles of samples analyzed after 0-, 1- and 4-hours of irradiation are shown here).

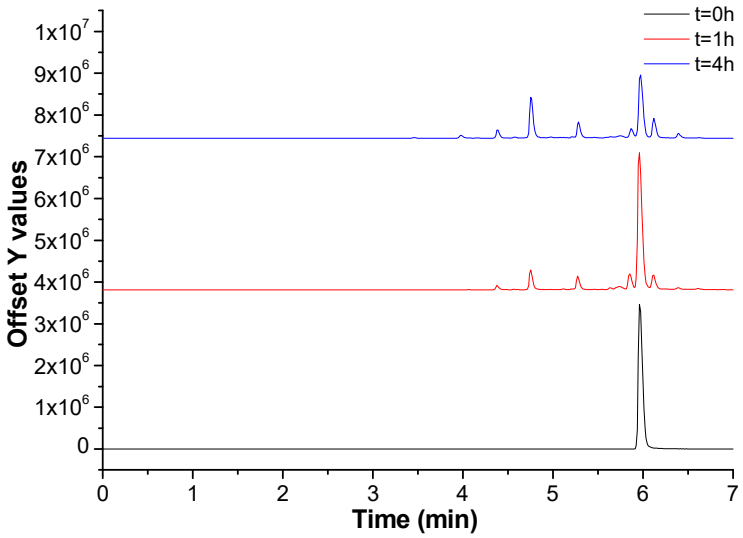

Figure 2. UHPLC-MS chromatogram (total ion current): formation of photodegradation products of irinotecan.

The intensity of the irinotecan peak at m/z 587 decreases with increasing irradiation time, whereas the emergence of new peaks at *m/z* values of 423, 437, 439, 529, 557, 573, 603 and 619 indicates the formation of possible photodegradation products of irinotecan. The next step for the other drugs, topotecan, gemcitabine and mitomycin, will be the development and validation of LC-MS/MS methods for the separation and identification of their respective photodegradation products as well as it was done for the irinotecan.

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CHEMICAL COMPOSITION OF ATMOSPHERIC WET AND DRY DEPOSITIONS IN THE VICTORIA LAND, ANTARCTICA

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Atmospheric depositions (typically, dry depositions of particles to the snow surface, wet depositions with snow or ice crystals, and occult depositions by fog and mist [1,2]) are the most important processes through which atmospheric pollutants (dust, particulate matter containing heavy metals, polycyclic aromatic hydrocarbons, dioxins, furans) are removed and transferred to terrestrial and aquatic ecosystems.
During the 2017-2018 Antarctic summer, the atmospheric depositions were sampled using bulk collectors (consisting of a polyethylene funnel connected to a bottle of the same material) placed in proximity of continental and coastal Antarctic sites corresponding to the complex net of weather stations that are part of the Meteo-climatological Observatory of the Italian Antarctic Programme (PNRA).
The samples were de-freezed and filtrated in order to separate the soluble and insoluble fractions of the atmospheric particulate. Cellulose mixed ester membrane filters were also weighed to determine the mass concentration of the particulate matter.
The soluble and insoluble fractions of major elements (Al, Ca, Mg, K, Na, Fe) and trace elements (Pb, Cd, Cu, Zn, Ni, As, Hg, V, Se) were analyzed by AAS, DMA and IC. Soluble fractions were also analyzed to determine the Cl^-, NO_3^-, NH_4^+, PO_4^{3-}, SO_4^{2-}, CH_3COO^-, $HCOO^-$ and $CH_3SO_3^-$ concentrations by IC.
Preliminary results on the soluble fraction of some metals and major constituents give the following concentrations, reported as means (interquartile range): Hg 0.067 (0.056 – 0.075) ng L^{-1}, Na^+, 2.1 (0.34 – 3.4) µg L^{-1}; K^+, 0.18 (0.055 – 0.26) µg L^{-1}; NH_4^+, 0.12 (0.009 – 0.037) µg L^{-1}; Mg^{2+} 0.33 (0.059 – 0.46) µg L^{-1}; Ca^{2+}, 0.26 (0.17 – 0.38) µg L^{-1}; Cl^-, 3.4 (0.31 – 6.6) µg L^{-1}; NO_3^-, 13.0 (0.16 – 0.77) µg L^{-1}; PO_4^{3-}, 0.088 (0.045 – 0.12) µg L^{-1}; SO_4^{2-}, 1.3 (0.34 – 1.4) µg L^{-1}; CH_3COO^-, 0.093 (0.044 – 0.083) µg L^{-1}; $HCOO^-$, 0.23 (0.070 – 0.21) µg L^{-1}; $CH_3SO_3^-$, 0.75 (0.016 – 0.10) µg L^{-1}.

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P93

POLLUTING METALS OF NEW GENERATION. VOLTAMMETRIC DETERMINATION OF PLATINUM GROUP METALS IN HERBAL MEDICINES

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Metals are ubiquitously in the environment, hence they are found in the food chain and in all the raw materials used for oral formulations and whatever may be ingested. Several metals may sometimes present high risks of toxicity to humans, even with irreversible effects. Some of them are toxic at any concentration, other become dangerous over a concentration limit. Hence, it is very important to determine metals down to trace and ultra-trace levels.
Herbal medicines/remedies are certainly an integral part of the food chain. These products are worldwide used every day by several millions of people for a number of reasons: nutritional, medicinal, but also to obtain performance-enhancing effects. Heavy metals have been found as ingredients in certain remedies. Unfortunately, due to the unregulated nature of the manufacture of herbal medicines, potentially lethal concentrations of these elements may occur. The International Organizations recommend that medicinal plants used as raw materials may be checked for the presence of toxic metals. Maximum permissible limits are set, paradoxically, only for mercury (1 ppm), lead (10 ppm), cadmium (0.3 ppm) and arsenic (10 ppm). A decision about the permissible limits for other metals has not been taken yet, because many of them are considered as micronutrients, sometimes even incorrectly.
In the last decade, high scientific attention has been addressed to platinum group metals (PGMs) in the environmental field, owing to their increasing concentration in all the environmental matrices. This is due to the increasing use of these metals in several fields, as for example production of industrial catalysts, anticancer drugs, jewels. In any case the cause of PGMs' growing concentrations in the environment is a consequence of the compelling employment of autocatalytic converters. Indeed, the incorrect "stop and go" use of these converters, with consequent deterioration and abrasion, certainly implies a considerable release in the environment of airborne particulate matter at high PGMs concentration, which results to be the greatest, if not the only, source of PGMs contamination of all the various environmental matrices, i.e. superficial water, soils, sediments, vegetables and so on. Really, after the first massive use of platinum, palladium and rhodium in the production of autocatalytic converters, there has been a gradual reduction of these PGMs with consequent growing use of iridium, ruthenium and, especially, osmium.
The present paper reports, for the first time, electroanalytical procedures for the voltammetric determination of PGMs ultra-traces by voltammetric techniques in herbal medicines, using a conventional three-electrodes cell. Precision and trueness, expressed as relative standard deviation and relative error, respectively, were generally lower than 7% in all cases. Once set up on the standard reference materials, the analytical procedure was

transferred and applied to commercial herbal medicine samples, and a critical comparison with spectroscopic measurements was done to evaluate the analytical performance.

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INVESTIGATION OF THE CHEMICAL CONTRIBUTION OF VOLCANIC AND FUMAROLIC EMISSION TO THE MARINE AEROSOL IN THE CENTRAL MEDITERRANEAN SEA: RESULTS FROM MED-OCEANOR 2017 CRUISE CAMPAIGN

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Volcanic emissions are an important source of atmospheric aerosol, whose contribution consists in the release of a considerable variety of compounds, such as water vapor, ash, CO_2, SO_2 and HCl [1] as well as trace metals and ionic species. These emissions have not simply a local impact on the troposphere but also a global impact, because the degassed compounds can reach the stratosphere [2]. However, nowadays there is a lack of information about the contribution of volcanic activity to aerosol levels, which significantly affects the assessment of environmental and human health risks. This gap of knowledge is of great concern in the central sector of the Mediterranean Sea owing to the many active volcanoes and solfatara fields. For this purpose, in the framework of the Med-Oceanor measurement program, a cruise campaign was carried out in summer 2017 to study the impact of the most important Mediterranean volcanoes on the level and composition of marine atmospheric aerosol.

The investigated area concerned 12 sites characterized by volcanic or fumarolic activity in the central Mediterranean sector, such as the Mount Etna, the Aeolian volcanic arc, Marsili Seamount, and the Phlegraean Fields. Aerosol particulate was sampled in its PM_{10} and $PM_{2.5}$ fractions and analyzed with different analytical techniques, to gather information about the concentration of major and trace elements, elemental carbon (EC), organic carbon (OC), and ionic species. We integrated the outcomes from the chemical characterization with data analysis tools including calculation of mass closure, enrichment factors, and factor analysis for the source apportionment (Figure 1). The results from chemical mass closure showed that average PM_{10} composition mainly comprised carbonaceous compounds and mineral dust, while carbonaceous material and secondary inorganic aerosol accounted for the major components of the $PM_{2.5}$ mass. In the volcanic areas, the use of triangular plots confirmed the interception of Etna and Stromboli plumes, while the enrichment factors showed the significant presence of elements associated to volcanic origins such as Tl, Cu, La, and alkali metals in accordance to previous studies [3, 4]. As regards the fumarole areas, the SO_2 concentrations, which represent a marker of geothermal activity [5], showed a strong correlation with SO_4^{2-} concentrations in both fractions of PM. Factor analysis allowed for the identification of six potential sources belonging not only to natural sources such as marine

and geogenic emissions but also related to the contribution of anthropogenic activities, such as combustion processes, vehicular traffic, and shipping emissions. Furthermore, stepwise linear discriminant analysis (S-LDA), which was used to seek for a model capable to discriminate between the sample of volcanic and fumarolic origin, pointed out the elements that discriminate the most between these two classes namely Sm, Hf, and Zr for volcano samples and Zn, Ca and Bi for solfatara sample.

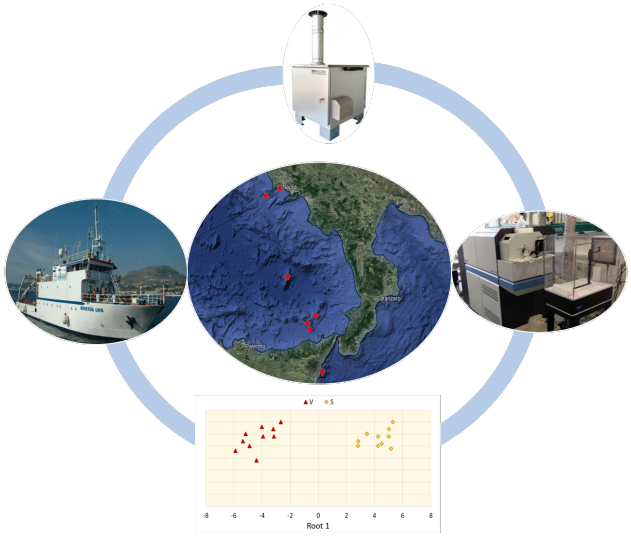

Figure 1 Graphical Abstract

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P95

Harpalus (Pseudophonus) rufipes (DE GEER, 1774) AS A BIOINDICATOR FOR DETECTING ENVIRONMENTAL CONTAMINATION: A PRELIMINARY STUDY OF HEAVY METAL POLLUTION IN CROP FIELD

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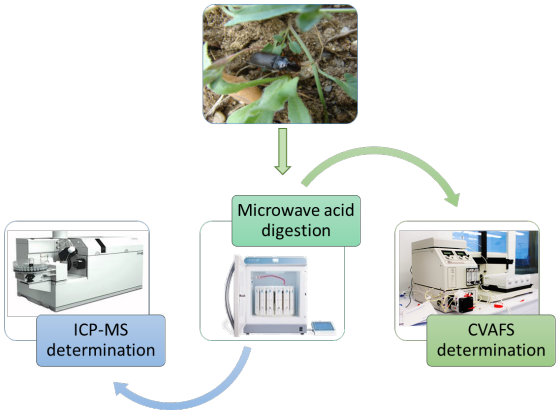
The metal concentration in the fertilizers that are commonly employed in croplands is rather low and often it is not thought as a considerable contributor to environmental contamination. However, the renewed use of fertilizers over the years may lead to the accumulation of metals in the receiving soils and their transfer in the food web with consequent relevant risks for human and ecosystem health [1]. Primary and secondary consumers and decomposers including soil invertebrate such as nematodes, earthworms, collembolan, isopods, spiders and insects can be useful accumulation indicators of environmental metal pollution [2, 3].
The aim of this study is to assess the level of metals in fertilized soils and their bioaccumulation in a secondary generalist predator, *Harpalus (Pseudophonus) rufipes*, to evaluate the transfer along the food chain in agroecosystems. For comprehensive monitoring, we investigated the concentration of metals in samples of non-fertilized soils used as a control, fertilized soils, and beetles as well as the conductibility and the pH of the soil samples. In the monitored sites (two fields approximately of 5 ha; non-fertilized: 39°17’10.28"N, 16°42’28.33"E, 1150 m a.s.l.; San Giovanni in Fiore, Calabria, Southern Italy; fertilized: 39°16’58.05"N, 16°38’43.26"E, 1240 m a.s.l.), a typical crop rotation was spring potatoes, wheat and lettuce in three years. Soil cores (n=6) were taken to a depth of about 15 cm from each field. Metal accumulation in carabids (BAF) was estimated as the ratio between the metal concentration in the beetle body and that in the soil.
We performed the sample preparation for the multielement analysis using microwave-assisted digestion of the soil and insect specimen with a mixture of HNO_3 and H_2O_2, with the addition of HF for the soil samples. Afterward, the instrumental analysis was performed by ICP-MS system. For mercury, given the low concentrations in the samples, we set up an analytical method for the quantification of its total content (THg) based on the protocol EPA 1631, which provides for the use of cold vapor atomic fluorescence spectroscopy (CVAFS). The THg concentrations were determined in the digested samples through the preliminary oxidation by BrCl to convert all mercury species to Hg^{2+} in solution, and later on, the addition of a reducing agent to generate volatile Hg^0 which was detected CVAFS. To avoid mercury

contamination, special care was taken during the sample preparation and the blank samples were strictly controlled.
The results showed a significant level of Be, Na, Pb, Cs, Se, Ga, Cr, Fe, Co, Ni, Zn, Cd, Tl, Ce and U in fertilized soil compared with control ones. A higher level of rare-earth metals was found in fertilized soils. The values of BAF indicated an accumulation of metal in beetles from the fertilized site (n=15) in the following rank order: Cd >Cu >Zu >Mg>K >Rb >Ni >Hg.
To conclude, this preliminary study indicated that the use of fertilizers may cause an accumulation of rare-earth metals in cropland soil. Moreover, the level of metal accumulated in beetles indicates that this species has a high detoxification capability. However, it is one the most frequent epigean species in agricultural ecosystems [4, 5] and it is well known to be involved in the pest control as predators of seeds [6] and invertebrates [7]. Thus, further study will confirm this species as a good indicator of metal pollution.



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P96

A NEW GREEN METHOD FOR THE QUANTIFICATION OF BENZOTHIAZOLES, BENZOTRIAZOLES AND BENZOSULFONAMIDES IN AIRBORNE PARTICULATE MATTER BY MICROWAVE-ASSISTED EXTRACTION COUPLED WITH SOLID-PHASE MICROEXTRACTION GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Benzothiazoles (BTHs), benzotriazoles (BTRs) and benzosulfonamides (BSAs) are high production volume chemicals. Since they are used in several industrial and household applications, it is expected their occurrence in various environmental matrices, especially water and air. These compounds could be considered as new emerging pollutants because of their health concern and the lack of information about their presence in outdoor air samples. Indeed, in literature, only a few recently published papers address their quantification in airborne particulate matter [1–4].
In this work, a new analytical method for the simultaneous quantification of BTHs, BTRs, and BSAs 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) (Figure 1) [5]. Fourteen analytes representative for the three classes of compounds were taken into account i.e., benzothiazole (BTH), 2-methylbenzothiazole (2-MeBTH), 2-(methylthio)benzothiazole (2-MeSBTH), 2-aminobenzothiazole (2-NH2BTH), 2-hydroxybenzothiazole (2-OHBTH), 2-mercaptobenzothiazole (2-SHBTH), benzotriazole (BTR), 4-methyl-1H-benzotriazole (4-MeBTR), 5-methyl-1H-benzotriazole (5-MeBTR), 5-chlorobenzotriazole (5-ClBTR), 5,6-dimethyl-1H-benzotriazole (5,6-MeMeBTR), benzenesulfonamide (BSA), p-toluenesulfonamide (p-TSA) and N-ethyl p-toluenesulfonamide (N-Et-p-TSA).

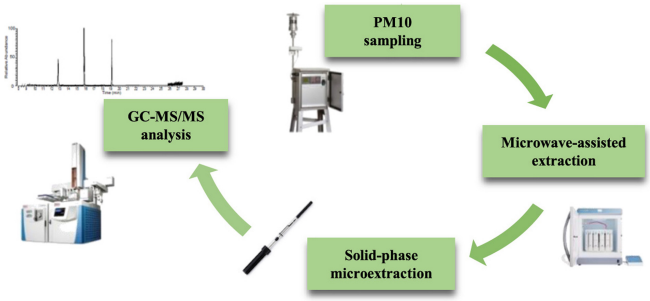

Figure 1 Graphical abstract of the proposed method

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Usually, BTHs, BTRs, and BSAs have been extracted from particulate matter by using traditional approaches such as solid-liquid extraction [2,4] or more recently pressurized liquid extraction systems using toxic organic solvents [1,3]. In our method, the BTHs, BTRs, and BSAs 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^{6-2} fractional factorial design was performed 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 and the factors affecting the SPME extraction (i.e., extraction time, extraction temperature and ionic strength of the sample) were optimized by a multivariate approach. The optimal working conditions were determined by using Derringer's desirability function. The analyte quantification 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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ACTIVITY RECYCLING OF WASTE AUTOMOBILE TIRES: TRANSFORMING CHAR IN OXYGEN REDUCTION REACTION CATALYSTS FOR ALKALINE FUEL CELLS

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In recent years, the fossil sources dependence of our society for both fuels and essential raw materials and the global warming have led to an increasing use of renewable sources and waste valorization processes. Nowadays the outstanding increase in the number of vehicles worldwide is among the most environmental problem because of the emission of harmful pollutants and the solid wastes disposal, in particular the removal of the used tires. There are several technologies for tires recycling. Thermal treatments may be used such as pyrolysis: a thermal decomposition process performed at higher temperature in an inert atmosphere which allows the transformation of complex substances in simple molecules. Among several heating technologies and apparatus used in pyrolysis process, microwave assisted pyrolysis (MAP) attracted attention, in recent years, due to the considerable advantages of this technology over conventional pyrolysis process [1]. It was observed that char obtained from microwave assisted pyrolysis of waste tires showed an interesting electrocatalytic activity in the Oxygen Reduction Reaction (ORR) in alkaline medium. ORR is regarded as one of the most important electrocatalytic reactions in electrochemical energy conversion system such as fuel cells and several industrials process. In order to speed up the ORR kinetics to reach a practically usable level in fuel cell, a cathode ORR catalyst is needed. Platinum-based materials are the most practical catalyst. To remove and replace platinum with less expensive materials it was proposed to exploit a synergic mechanism with one metal able to break the O-O bond of molecular oxygen and second metal capable in reducing the adsorbed oxygen so formed. The presence of specific metals together with a high carbon content are essential requirements for catalysts for ORR [2]. The effect of further calcination temperature (no Map) on ORR was investigate. The results show that calcination temperature effect on ORR performance. Notable, electrochemical analysis of CH_@4_450° catalyst showed both onset potential (Eon) and electrons number exchange for O2 molecule similar to Platinum electrode.

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P98

CHEMICAL CHARACTERIZATION OF PARTICULATE (PM10) OF DIFFERENT SITES ON SICILY TO EVIDENCE SHIP EMISSION.

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In a general contest to analyse the particulate (PM10 and 2.5) from different sites of Sicily, we begun a study to extend the monitoring of trace elements contained in atmospheric aerosol to rare earth elements (REEs), La and Ce in particular, and V and Ni. Concentrations of La and Ce, were used to identify possible contributions from refineries, whose emissions are also characterized by elevated V and Ni amounts. At the same time, we have extended the current knowledge on the composition of particulate through the measurement of Na, K, Ca and Mg in order to characterize sea salt aerosol. Also, the aim of the study, with the selected elements analysed and sampling carried out in harbour or very close to sea, was to evidence the role and the contribute of ship emission to particulate[1].
As regards the sampling and analysis method, the guidelines of current legislation (UNI EN 14902:2005) that provides for the sampling of the fraction of PM10 particulate, have been followed. Particulate matter with aerodynamic diameters lower than 10 μm, (PM10) were daily collected during the period November 2018 – June 2019 sampling for 24 hours 50 m^3 of air on quartz filter. Selected metals were determined after mineralization and ICPMS determination. Na, K, Ca and Mg were determined on filter after equilibration with ultrapure water for 24 hours. The result were statistically treated and discussed.
This study is the first carried out in Sicily and contributes to the identification and characterization of the emissions on the aerosol distribution.

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P99

MICROALGAE AS A SOURCE OF BIOACTIVE PEPTIDES: EXTRACTION, IDENTIFICATION AND ASSESSMENT OF THEIR ACTIVITIES

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The research in the field of food bioactive peptides has greatly increased in the last decades, due to their health benefits and functional and bioactive properties. Besides the classical matrices considered as sources of bioactive peptides (e. g. meat, fish, milk, and soybean) [1], recently, attention is being paid to microalgae. Microalgae are unicellular marine organisms that have promoted complex biochemical pathways to survive in greatly competitive marine environments; their chemical composition may vary according to environmental and growing factors. Indeed, microalgae contain significant amounts of high-quality proteins which could represent a source of novel bioactive peptides.
In this research [2], we developed an analytical methodology for the extraction, separation and identification of bioactive peptides in protein hydrolysates obtained from *Tetradesmus Obliquus* microalgae. Since microalgae are characterized by strong cell walls, to obtain the maximum yield in protein extraction, seven different protein extraction protocols, based on mechanical and chemical methods, were tested. The best protocol consisted in milling the tissue of *T. Obliquus* with glass beads. The protein extract was then subjected to enzymatic digestion with Alcalase®, and subsequently the hydrolysate was purified by two-dimensional semi-preparative reversed phase (RP) liquid chromatography (LC). Fractions were assayed for antioxidant and antihypertensive activities and only the most active ones were finally analysed by RP nanoLC-tandem mass spectrometry. Around 500 peptide sequences were identified in these fractions. The identified peptides were subjected to an in silico analysis by PeptideRanker algorithm in order to assign a score of bioactivity probability. Twenty-five sequenced peptides resulted to be potential candidates with antioxidant and angiotensin-converting-enzyme (ACE)-inhibitory activities. Four of these peptides, namely WPRGYFL, GPDRPKFLGPF, WYGPDRPKFL, SDWDRF, were selected for synthesis and tested *in-vitro* for the specific bioactivity, exhibiting good values of antioxidant and ACE-inhibitory activity.

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P100

STUDY OF BIOFILM FORMATION ON DIFFERENT MATERIALS USED IN DRINKING WATER DISTRIBUTION SYSTEMS (DWDS) AND EFFECTS OF DISINFECTION TREATMENT

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Microbial biofilms are commonly defined as sessile microbial consortia established in a three-dimensional structure. It consists of microbial aggregates where cells adhere to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS), extracellular DNA, proteins and polysaccharides. Microbial cells within biofilms acquire capabilities that enable them to withstand antimicrobial agents and chemicals. The major factors affecting the formation of biofilm on surfaces are the electrochemical properties of the surface, nutrient availability and water flow. Growth of microorganisms in water distribution system can be affected by both biological factors and interaction of various physico-chemical factors such as pipe material, water temperature, pH, hydraulic conditions, nutrients, and disinfectant concentration. Different types of pipe materials can affect microbial growth by releasing chemical compounds such as phosphorus ions, copper, iron and organic compounds.
Drinking water distribution systems contain microorganisms in both the flowing water and in biofilm that may be a reservoir for pathogens, plays a role in corrosion and impacts the aesthetic of the water.
Thus presence of biofilm in drinking water pipe networks can be responsible for a wide range of water quality and operational problems.
The aim of this study is to evaluate the development of biofilms produced by the bacterial species *Pseudomonas aeruginosa* on different materials (*e.g.*, PVC, PEAD, galvanized steel) used in drinking water distribution systems. These materials are placed inside glass tanks, previously sanitized, and filled with sterilized tap water inoculated with a known concentration of the bacterium, under static conditions, and in established thermal conditions (25°C). The biofilm analysis obtained on the surface of the different materials is carried out at time zero and subsequently once every two weeks, determining heterotrophic count (HC) by cultural methods, and adenosine triphosphate (ATP) by luminometer.
The impact of disinfection on the biofilms is finally evaluated by subjecting the growth biofilm to monochloramine treatment and measuring the effects of the disinfectant on this biological structure. The use of monochloramine is chosen since it is associated to a low formation of disinfection byproducts; moreover monochloramine was reported to penetrate better into ATP biofilms than chlorine, because it seems to have a lower capacity for reaction with biofilm constituents, proving to be a better disinfectant than chlorine.

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P101

DETERMINATION OF POLYSILOXANES IN SLUDGES BY ANALYTICAL PYROLYSIS

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Siloxanes comprise a large family of low and high molecular weight species used in a broad range of industrial, agricultural and consumer applications. The most common polymeric siloxanes (silicones) are linear poly(dimethylsiloxanes) (PDMS, named as dimethicone in some commercial products) and copolymers bearing other side chains (e.g. PEG, poly(ethylene glycol)). PDMS does not present recognized environmental and health risks, while cyclic volatile methyl siloxanes (cVMS) are a matter of concern [1]. For instance, decamethylcyclopentasiloxane (D_5, D=(Me_2SiO)) is considered very bioaccumulative and persistent in natural waters and sediments; it is released by direct use as intentional constituent (e.g. cosmetic ingredient) or impurity in silicones. Analytical methods of silicones in environmental matrices were few and focused to PDMS [2]. The most commonly used were based on silicon-selective techniques, such as ^{29}Si-NMR and ICP-AES, the latter combined with GPC or HPLC. The main drawback was the interference by naturally occurring silicon species, such as humic matter-clay aggregates. Analytical pyrolysis (Py) combined with GC-MS is a powerful technique for the analysis of polymers which has been applied to the identification and quantitation of microplastics in the environment. This study aimed at evaluating the potential of Py for the determination of silicones. The attention was focused to sludge from wastewater treatment plants which are a possible source of siloxanes in the environment. Commercial siloxanes were pyrolyzed under different conditions to identify prominent pyrolysis products and select optimal analytical conditions. In a typical procedure, *n*-hexane extracts of dried sludge added with internal standard (tetrakis(trimethylsilyloxy)silane) were flash pyrolyzed off-line at 700 °C (set temperature) by a resistively heated platinum filament for 90 seconds under 50 ml min^{-1} nitrogen flow; pyrolysis products were sampled by SPME onto a PDMS-DVB fiber and thermally desorbed in the inject port for GC separation by a polar stationary phase and detection by QMS. The pyrograms of different silicones were dominated by cVMS from D_3 to D_8 with a distribution reflecting sample type and fiber selectivity [1]. Thermal degradation products of side chains were detected at lower amounts and tentatively identified only in the case of PEG derivatives. Pyrograms of sludges from industrial and municipal wastewater treatment plants were dominated by aliphatic and aromatic hydrocarbons originated from the thermal degradation of lipids. Procedural blanks and calibration with dimethicone indicated quantitation possible in the microgram range which resulted adequate for the analysis of polysiloxanes in sludges.

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QUALITATIVE AND QUANTITATIVE DETERMINATION OF SOME ORGANIC MOLECULES USING A DMFC DEVICE AND CHEMOMETRICS

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Making use of a small commercial Direct Methanol Fuel Cell (DMFC), used as an analytical sensor [1] and chemometric methods, organic molecules very different from one another, practically having in common only one –OH group, can be determined not only quantitatively, but also qualitatively. In this communication, the following 7 organic molecules were considered: methanol, ethanol, propanol, chloramphenicol, imipenem, atropine and cortisone; that is, 3 alcohols, 2 antibiotics and 2 molecules very important from the bio-pharmaceutical point of view. From a quantitative point of view, the traditional approach was followed: seven different calibration curves were built (one for each molecule), which show very different calibration sensitivity. Each of them allows, therefore, the quantitative determination of the corresponding organic molecule, even if with very different sensitivities among them. For the qualitative analysis of single molecules, the approach has been much more innovative. In fact, by processing the data from each of the individual curves obtained through the fuel cell, which represent the sensor's response to each of the molecules considered and processing them using chemometric methods, it is possible to directly identify and recognize each of the 7 organic molecules.

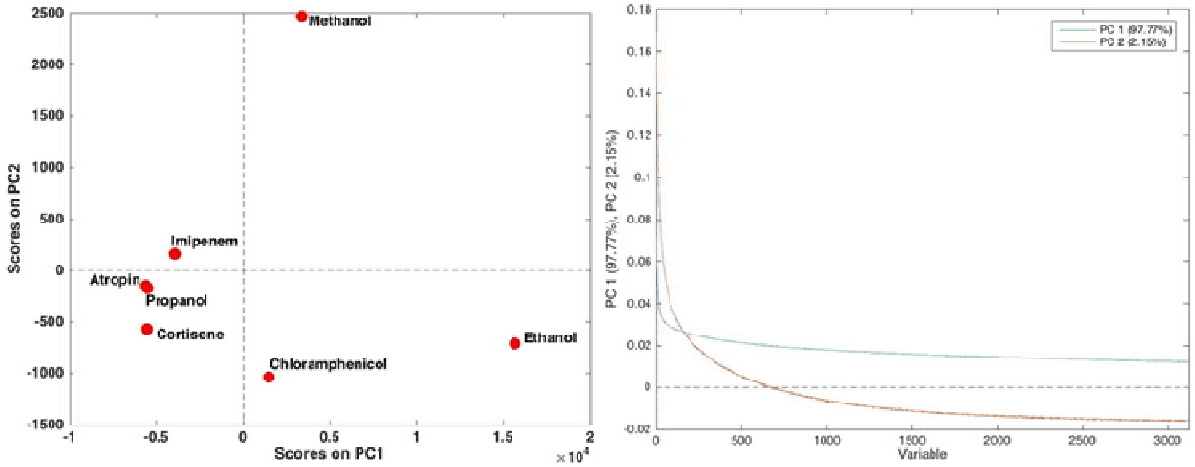

Figure 1. Results of PCA on the analyzed "response" data set: (left) scores plot; (right) loading plot.

The loading plot seems to indicate that there are two kind of exponential decays characterizing the analytes, one (contributing the most to PC1) which is faster, and a second one (more relevant for PC2) which is slower. By comparing these outcomes with the corresponding scores plot, one could infer that in ethanol and, to a lesser extent methanol

and chloramphenicol, are characterized by a higher contribution of the faster component, whereas the slowest one contributes the most in particular for methanol.
We also tried to use data from calibration curves, which show different slope values, for each of the 7 organic molecules considered, performing PCA also on them. In Fig 2 we can observe the "scores" representation, along the first two principal components. The representation is consistent with the sample distribution observed in figure 1, and confirms, once again, a difference in behavior between methanol and the other six substances.

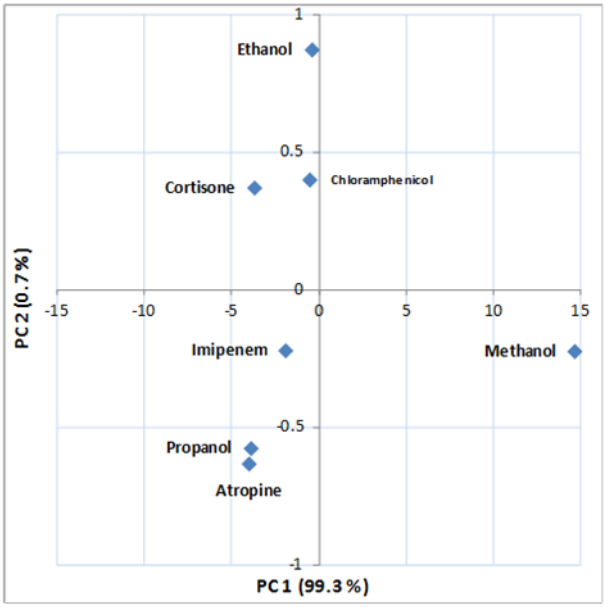

Figure 2. Results of PCA on the analyzed different calibration sensitivity data. Scores plot.

Lastly, in order to have a better insight into the characteristics of the systems, the data were further analyzed by power-slicing followed by PARAFAC data processing [2].
The results highlight how in general the current drop is higher for ethanol, methanol and chloramphenicol, but also that, for these molecules, the slower decaying components are more relevant. On the other hand, the other molecules show a lower overall intensity drop and, when looking at the individual contributions, a higher amount of the faster decaying components.
In conclusion, the results obtained from this first approach, based on the use of a DMFC cell type sensor and chemometric methods, seem innovative and promising in the light of further and wider developments in the qualitative and quantitative analysis of several other organic molecules.

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INVESTIGATION ON URBAN RAIN POLLUTION, A CASE OF STUDY: RAIN OVER ROME

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The composition of rainwater is the result of complex interactions between the water vapor of the clouds, the dust raised by the wind and the atmospheric gases. The chemical composition of rainfall can probably be related to local sources of pollution in urban areas, but also to relatively distant sources, provided that there is an atmospheric transport phenomenon.
The pH of rainfall depends essentially on the result of neutralizing reactions between the acid components of rainwater [1], mainly derived from acid gases such as carbon dioxide, nitrogen oxides and sulphur oxides, and the basic components, mainly ammonium, bicarbonates, carbonate and hydroxides of calcium and magnesium. The latter coming mainly from erosion phenomena. The presence of other metals in the rains seems to be linked, in addition to erosion phenomena, also to their presence in fossil fuels which, during combustion, produce oxides which are then hydrated by the water in the cloud. This communication shows the results of the monitoring of chemical-physical data of rain samples fallen in Rome during 7 months of the year 2018.
Data that should have a great interest given the large number of monuments and archaeological finds exposed outdoors in this city. Despite the importance of the subject there are no major studies published recently on the rain that fell on Rome since 1971 [2]. The present experimental study seeks to fill this gap, at least in part. For this purpose 61 rain samples were collected between January and July of 2018, the rain was collected on the roof of the Department of Chemistry of the University "La Sapienza". The sampled rain was poured into Falcon tubes kept at 4 °C, a part of the rain was used for immediate direct analyses, the other used for ion chromatography. The direct analyses were performed in the Falcons itself with bench top instruments and electrodes (by Vernier, USA) and covered the pH, conductivity, temperature and redox potential (ORP).
At the same time the data on speed and wind direction were acquired, in order to find a correlation between the variations in the composition of the rains and the ion transport phenomena [3], data obtained from the weather station of Piazza Galeria, about 4 Km from the sampling point and data on the volume of rain fell and its temperature were collected from the weather station in the Civil Engineering headquarters, in Via Mozambano, about 500 meters from the sampling point. The seasonal average of the data collected and experimentally obtained in this research is shown in table 1.
The principal components analysis (PCA) was carried out on the data collected using only the chemical-physical parameters, divided by seasons winter, spring and summer, pre-treating

the data with autoscaling, but omitting the data of the ionic concentrations that turned out to be redundant for this purpose.
The use of chemical-physical data alone has allowed the separation of samples between the different seasons. Scores have been reported in a 3D chart in Figure 1.

Table 1: seasonal average of the measured parameters, LOQ = Limit Of Quantification

Season	pluvio	acidity	ORP	conduct.	Na^+	K^+	Mg^+	Ca^{++}	Cl^-	NO_3^-	SO_4^{--}
	mm	pH	mV	µS	ppm	ppm	Ppm	ppm	ppm	ppm	ppm
Winter	7.5	6.21	347	42.5	2.5	1.6	1.3	8.0	3.5	2.9	4.0
Spring	9.3	6.57	344	48.5	4.0	2.6	2.7	7.6	5.4	3.3	4.1
summer	8.2	6.63	342	29.1	2.5	1.2	<LOQ	2.8	2.8	2.5	2.3

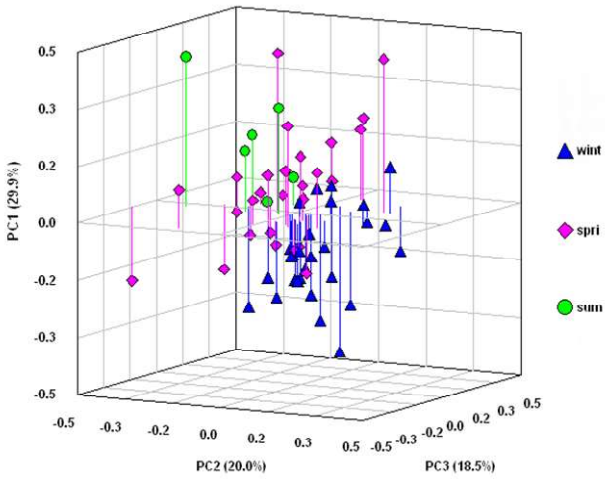

Figure 1. Graphic representation of the first 3 main components after dividing the data set of the rainfall in different seasons, using only the chemical-physical data and excluding the ionic concentrations

In conclusion, this research has shown that, even if there are no serious problems related to the acidity of the rain, there are in any case precipitation with a discrete content of nitrates and sulphates that can create problems for the numerous monuments and outdoor stone finds. Also at certain times of the year there is a probable effect of transport of nitrate and sulphate from the industrial zone to the east of Rome and sea salt from the Tyrrhenian Sea to the west of Rome.
Finally, there is a certain degree of correlation between rainfall, pH, ORP and conductivity compared to the seasons. On the contrary, the trend of concentrations of the main ions seems to be independent of seasonal variations. We are also urging the publication of data collected by various government agencies, to create a historical archive not only of rainfall, but also of its composition.

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FOOD WASTE AS BIOCOMPATIBLE AND INEXPENSIVE RENEWABLE CARRIERS FOR LACCASE IMMOBILIZATION

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Over the last decades, agricultural-food waste have become an increasing concern. In fact on the basis of the information from the European Union (EU), the total EU food waste in 2012 was approximately 88 million metric tons, where more than 72% of the production belonged to households [1]. In particular, over 10^{12} eggs are consumed worldwide and their by-products (shell and membrane) have generally been overlooked and disposed as waste. Moreover billions of tons of lignocellulose wastes, are also produced worldwide as residues from agricultural activities and industrial food processing. The most common one is spent grain, that is a brewing by-product, corresponding to around 85% of total by-products generated. According to Pospikova and Safarik (2012)[2], spent grain accounts, on average, for 31% of the original malt weight, representing approximately 20 kg per 100 L of beer produced.

Food waste are usually burned and they undergo improper storage in fact for this reasons they may cause significant environmental problems. One solution is to recycle them as an alternative useful materials.

An immobilized enzyme is proven to be more efficient than its free form because can enhance the enzyme stability, activity and recovery, leading to significant economic benefits in industrial application. One of the most important factors in enzyme immobilization technology is the support material, because its characteristics determine the performance of the complex enzyme-support. Nonetheless high cost of commercial supports is encouraging searches for cheaper substitutes. In this context the use of food waste as enzymatic supports could be a valid alternative, both for reducing their disposal problems and for a significant economic benefits, which guarantee the application of enzyme on a large scale in food technology or biotechnology.

Thus, the aim of this study is to develop a suitable method for laccase from *Trametes versicolor* immobilization on egg shell membrane (ESM) and on spent grain (SG) which have large surface areas and porosity. These peculiarities permit a high enzyme loading and guarantee a high protection of the immobilized protein from the environment.

Laccases, which are 'ecofriendly' oxidoreductases that produce water as the only by-product, catalyze the oxidation of a broad range of substrates and consequently can be employed for a great variety of biotechnological, environmental and industrial applications. Eggshell membrane (ESM) is a complex structure situated between the eggshell and the egg white with inert nature and water insolubility. It is mainly made up of polysaccharides (5%) and natural proteins (95%) [3] while spent grain contains about 17-25% cellulose, 21-28% non-cellulosic polysaccharides, 12-28% lignin, 15-24% protein and less than 11% lipid [4].

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Both of waste (ESM and SG) contain a high number of functional groups on the surface suitable for laccase covalent linking. Two different immobilization protocols were employed for ESM. The first one was performed by modifying the method reported by Pundir et al., 2009 [5] for glucose oxidase, in order to eliminate cross-linking agent. For this reason the periodate-oxidized laccase was directly linked on ESM activated with $NiCl_2$. The second one was based on the cross-linking reaction between the same terminal functional groups of the protein and the reactive groups on the ESM. So glutaraldehyde was dropped onto the ESM surface in order to form the Shiff bases with the amino groups of the eggshell membrane and of the laccase.

For laccase immobilization on spent grain, instead, a stepwise pretreatment of SG with HCl and NaOH for the hydrolysis of residual starchy endosperm and delignification was performed. In acid hydrolysis the hemicellulose is partially solubilized, exposing a larger area of cellulose to the enzymatic attack. Addition of alkali (0.1 M NaOH) is responsible for removing lignin forming a material with reduced crystallinity of cellulose.

Finally, a comparison of specific activity (U/g) and reusability of laccase immobilized on BSG and ESM was performed in order to evaluate the best promising biocatalyst which can be used in future biotechnological application.

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A FORS (FIBER OPTICS REFLECTANCE SPECTROSCOPY) DATABASE FOR THE RE-EVALUATION OF A HISTORIC PATTERN BOOKS COLLECTION: THE CASE OF THE *CASSELLA COLOR COMPANY*

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The *Cassella Color Company* was founded at Frankfurt am Main (Germany) by Leopold Cassella in 1789 and started towards the end of the 19th century a dyestuff manufacture, rapidly becoming one of the worldwide major synthetic dyes producers. In particular, the firm specialized in the patenting and production of "Diamine Colors", azo dyes with the general structure of a diamine (benzidine, tolidine, methoxy tolidine or ethoxy tolidine) linked to two terminal alike or different groups [1]. Their pattern books describe the developing of these dyes and the various dyeing processes of silk, cotton, linen, wool, straw and chips: they contain also fragments of fabrics colored with the synthesized dyes, thoroughly supplied with the description and percentage of each dye, applied individually or in a mixture.
The precious collection of the Commodity Science Museum of the University *Aldo Moro* of Bari (Italy) includes rare pattern books of yarns and textiles dated to the end of the 19th – beginning of the 20th century, among which an important corpus of the *Cassella Color Company* editions.
This work is included in a wider project aimed to the re-evaluation of the Commodity Science Museum collection, starting with the scientific cataloguing by means of reflectance spectroscopy data of the dyestuff production from the above-mentioned firm.
Fiber Optics Reflectance Spectroscopy (FORS), much used for the identification of dyes and pigments, has scarcely been employed in the study of textile dyestuff [2], for which the main applications were directed to the revelation of natural/traditional dyes in fabrics prior to the era of synthetic colors [3, 4].
The FORS technique was employed with the aim of generating a reference database for the future characterizations of 20th century dyestuff. Up to now, silk cotton blend, wool cotton blend, straw and chips dyed with Diamine Colors and finally color lakes precipitated in different ways were analyzed.

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A NON-INVASIVE PROTOCOL FOR IN SITU IDENTIFICATION OF PROTEIN BINDERS IN PAINTING SAMPLES

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The identification of the medium used (i.e., organic binder) in artworks and polychromies can provide relevant hints to design the proper restoration work [1]. Protein-based mediums such as animal glues, casein and egg white or egg yolk are among the oldest binders used in painting [2][3]. Currently, the identification of protein binders is currently carried out by removing minute quantities of sample and proceeding with the classic bottom-up proteomic approach. It consists of analyzing the peptide mixture resulting from protein hydrolysis (commonly with trypsin) either by peptide mass fingerprinting (PMF) or by peptide sequencing using tandem mass spectrometry (MS/MS). When applying PMF, protein identification relies on accurate measurements of peptides molecular weights, whereas for MS/MS peptides are fragmented in the source or in the analyzer of the mass spectrometer and amino acid sequences are obtained by the elucidation of fragment ions resulting from the fragmentation [4].
The examination of precious and unique objects, especially in the cultural heritage field, deserves the use of non-invasive or minimally invasive procedures and techniques in order to preserve their content. The usually employed non-invasive approaches such as multivariate analysis of combined Raman and fibre-optic reflectance spectra like visible reflectance spectra can give preliminary results [5], but mass spectrometry is still required for establishing the right identity of protein binders. Recently, a new method for the minimally invasive analysis of proteins has been proposed by using cellulose acetate sheets as a support surface for fungal proteins Vmh2 hydrophobin to immobilize trypsin [6]. Sheets of modified cellulose large 2x2 cm were used for protein digestion of several types of supports and investigated by matrix-assisted lased desorption/ionization (MALDI)- time of flight (ToF) MS. Starting from this idea, we proposed a very simplified protocol for in-situ digestion of proteins without the need for sampling and without the use of organic solvents or special pretreatments as well. Using a small hydrophilic gel (2x2 mm), previously loaded with a trypsin solution, the proteins occurring in the small pictorial contact area are digested. Paint replicas were made on 76×26 mm glass slides by applying mixtures of egg yolk, milk casein, bovine and rabbit collagen binders with historical pigments as red bole, white lead and ultramarine (Figure 1). Moreover, aged samples (up to four years in presence of light) were also examined.

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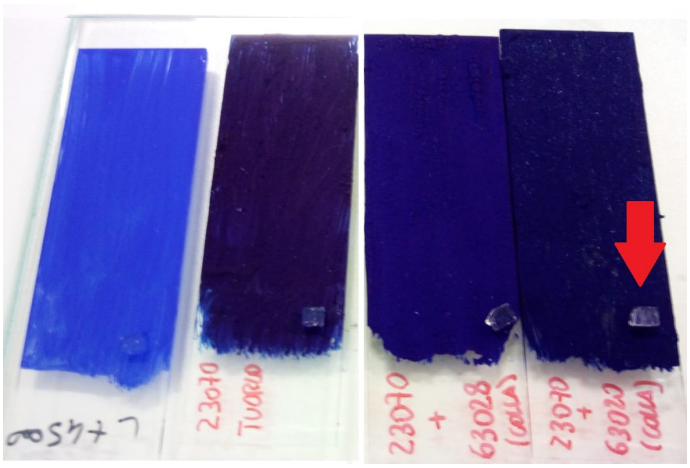

Figure 1. Paint replicas made with different protein binders (milk, egg yolk, rabbit and bovine collagen) and various blue pigments.

From light microscope pictures it was observed that the treated paint layers are not perceptibly altered upon the gel contact. The peptides released directly on the gel can be identified by reverse phase liquid chromatography coupled by electrospray ionization and MS or by MALDI MS/MS. During the optimization process, a few experimental conditions were changed such as the trypsin loading concentration, gel size and the contact times. Apparently, the suggested protocol represents an innovative step forward for the use of a minimally invasive tool in the proteomics field of cultural heritage specimens.

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EXTENSIVE PREVENTIVE DIAGNOSTICS FOR BIOCLEANING AND BIOCONSOLIDATION OF TWO RUPESTRIAN CHURCHES

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Historical-artistic heritage suffers biotic and abiotic degradation more than in the past due to the increasing environmental pollution.
The process of deterioration is progressive and irreversible, and the timing and mode of impact is different depending on the characteristics of the monument (location, orientation, mineralogical and structural properties), microclimate (temperature, humidity, solar radiation, wind regime, precipitations), air pollution (particulate, concentrations of SO_2, NOx, CO_2), presence of specific flora and fauna on the surfaces. The conservation and recovery of such structures require the understanding of physical-chemical and biological processes causing the deterioration of materials, and the knowledge of restoration strategies [1].
The characterization of structure and microstructure of historical materials is mandatory in preventing, and eventually recovering, degradation effects. Ideally, the analysis of an artwork should be complete, efficient, rapid and, if possible, non destructive when dealing with precious or unique objects. Thus, measurement should be carried out in-situ or by removing very small amounts of material. XRD, XPS, GPR, MGE and FT-IR are some techniques widely applied to study materials used in historical or artistic artefacts.
Complying with this purpose, two Rupestrian Church (*Santa Lucia alle Malve* and *San Pietro Barisano*) located in Matera town in the area named "SASSI", rich of natural vegetation and biodiversity, were studied in order to carry out a possible restoration, by using plant secondary metabolites, and bio-consolidation, by using calcinogenic bacteria.
Chromatic alteration of the stone surfaces, presence of patinas, sagging, efflorescence and detachment of some portions of plaster and a lush biological colonization were observed, favored by water infiltrations and by particular internal microclimatic conditions.
Sampling and characterization of the stone supports were performed. The detached rock fragments were subjected to petrographic and surface analysis by using Xray diffraction technique, SEM - EDS system and X-ray photoelectron spectroscopy(XPS).
All analytics showed an advanced state of decay with the formation of degradation products such as sulfates and calcium nitrate. Moreover, thermo-analysis has confirmed the presence of water on the top and in the wall of the churches structure caused by outside fractures. Glycoalkaloids (secondary metabolites extracted from *Solanaceae* plants) [2,3] were used for the bio-cleaning. Autochthonous colonizer bacteria found on the building surfaces were used for the stones' bio-consolidation in situ.

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P108

ARCHAEOMETRIC STUDY OF ROMAN MOSAIC TESSERAE FROM THE ARCHAEOLOGICAL AREA OF VILLA SAN PANCRAZIO (TAORMINA, ITALY): ED-XRF AND RAMAN SPECTROSCOPY ANALYSES FOR DATING AND MANUFACTURING ASSESSMENT

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Villa San Pancrazio is one of the most extensive and significant archaeological areas of the city of Taormina (ancient Tauromenion / Tauromenium). The excavations in progress since 2015 are bringing to light a vast multi-layered Roman-Imperial residential quarter featuring luxurious dwellings decorated with wall paintings and mosaic floors. The present work deals with the study of tesserae sampled from polychrome and black and white mosaics, generally dated back to the Middle Imperial period, between the II-III century A.D. Mosaics are difficult to be precisely dated since stylistic and iconographic criteria are usually insufficient for a reliable chronological and manufacturing attribution [1]; thus this archaeometric investigation is meant to provide a scientific support for materials dating, by means of their identification as well as their production techniques assessment, that can also give some clues on the economic and cultural exchanges [2]. With this aim, a non-destructive multianalytical methodology, based on the combination of X-ray fluorescence by energy dispersive (ED-XRF), Raman spectroscopy and X-ray diffraction analysis (XRD), was used to characterize a wide selection of stone, ceramic and glass tesserae collected from the different mosaics. In addition, chemometric tools were used to extend the information about some raw materials origin and supply, by means of Principal Component Analysis (PCA) and class modeling tools. Soluble salts determinations by Ionic Chromatography (IC-HPLC) were also carried out to ascertain the conservation state of the buried samples.
The investigations provide the establishment of the natural lithotypes used and how the artificial materials, i.e. ceramics, transparent and opaque glasses, were produced. The data comparison with the ones coming from other archaeological sites of the same age allowed the specific identification of coloring and opacifying agents applied, as for example, Co^{2+} and

both the hexagonal $CaSb_2O_6$ and the orthorhombic $Ca_2Sb_2O_7$ calcium antimonate phases in the opaque blue samples, respectively (Fig. 1) [3].

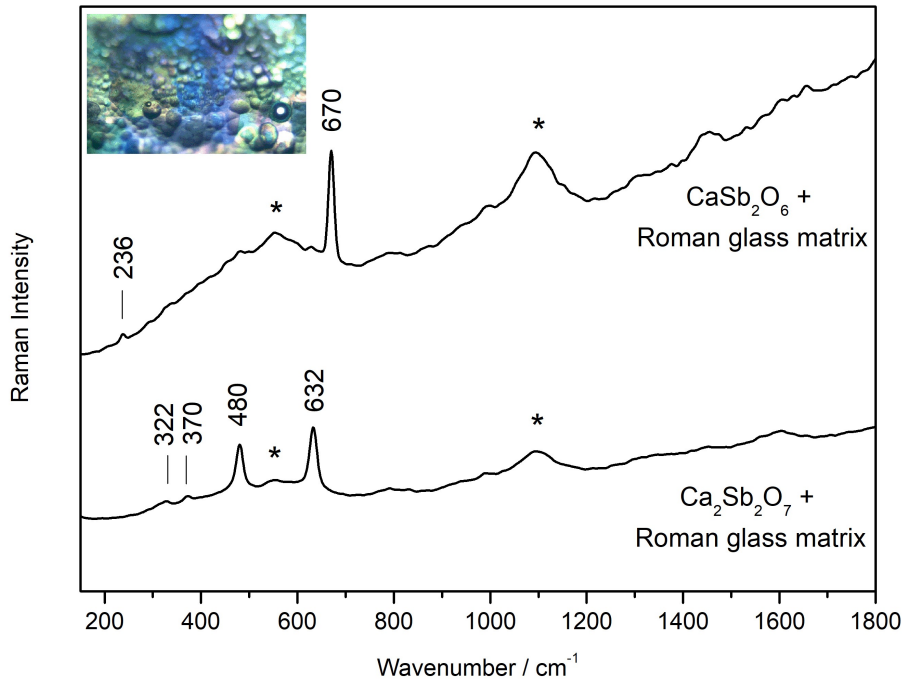

Figure 1. Raman spectra collected from blue opaque tesserae, in which $CaSb_2O_6$ and Ca_2Sb2O_7 calcium antimonate signals were identified inside a Roma glass bead matrix (*).

Data so far acquired allowed to ascertain the significant technical and qualitative level of the mosaic systems of the residences, thus confirming the high socio-economic profile of the owners. Preliminary results on soluble salts determination suggest that the tesserae are not involved in significant degradation phenomena.

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ARCHAEOLOGICAL MATERIALS FROM GABII (CENTRAL ITALY): KNOWLEDGE OF OFFERINGS AND RITUALS AT THE INFANT BURIALS THROUGH AN INTEGRATED APPROACH

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The ancient Latin city of *Gabii* is situated 18 km (11.2 miles) to the east of Rome (Central Italy) along the modern Via Prenestina. *Gabii* was a renowned city in Roman times, particularly during the Republican period and there are various influences in the site that can be identified in Roman culture itself. *Gabii* is also one of the most significant and important archaeological sites in the territory of the Municipality of Rome and due to its characteristics, it represents today an extraordinary research context. From the excavations carried out in the past it is possible to see how, under the soil, the main structures and buildings of the ancient city are still largely preserved. Among the various testimonies of the past, the tombs, and the micro and macro remains that these contain, represent an opportunity to investigate such practices in the context of Early Iron Age and Orientalizing Latium. In particular, the finds from the Area D baby burials of *Gabii* enriched the existing dataset so far significantly, allowing us to explore funerary ritual behavior in a more systematic way.

This work reports the results of the detailed examination of four tombs (Tombs 30, 50, 51 and 52) of archaeological site. The field strategy for the excavation of the tombs was geared from the start towards both the systematic retrieval of archaeobotanical and zooarchaeological remains and the sampling for organic residue analysis. Aiming for total recovery, the sediments from the tomb fills were sifted in their entirety as their stratigraphic excavation progressed, and samples were taken for flotation. This careful screening allowed for the detection of concentrations of organic material that represent plant and/or animal depositions. The excavation and removal of the grave goods was carried out following strict protocols for residue sampling, minimizing the risk of organic contamination. Samples were analysed by High Temperature Gas Chromatography/Mass Spectrometry (HTGC/MS) and Gas chromatography/Combustion/Isotope ratio mass spectrometry (GC-C-IRMS). For each burial, a subset of vessels including both closed and open shapes was selected, such as cups, open bowl without foot, amphoretta, amphora with dots, Kantharos, plate on a foot, olla, and olpe in bucchero.

The results demonstrate the still largely unexploited potential of this sort of integrated studies, encouraging us to expand the application of chemical methods to contexts from other well–controlled excavations.

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FIRST EVIDENCE OF *OPUNTIA FICUS INDICA* LEAVES USED AS SURFACE FINISHING TREATMENTS ON THE PIETRA LECCESE

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Lecce stone belongs to the group of Miocene limestone and is distributed in many areas of the Salento peninsula. The great part of the historical buildings, both religious and civil, in this area have been constructed with this porous and soft material.
In the past, various methods and recipes have been used to protect the surfaces and ensure their longevity. However, these ancient "recipes" are unidentified, because these techniques were known only by the artisans who used them [2].
For this purpose, selected religious and civil buildings in the territory of the province of Lecce, made of Lecce stone, not yet restored and possibly treated with some of these unknown recipes because of their good conservation state, were studied and the presence of surface treatments was investigated.
Samples were analysed by Py/GC-MS with and without thermally assisted hydrolysis and methylation using tetramethylammonium hydroxide (TMAH). The results of the analyses showed that these buildings have been protected with different natural products. Moreover, for the first time, chemical biomarkers demonstrating the use of prickly pear leaves (Opuntia ficus indica [3]) as protective surface finishes have been identified.

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A MULTI-ANALYTICAL DIAGNOSTIC APPROACH FOR THE CHARACTERIZATION OF HEAVILY REPAINTED WOODEN SCULPTURES DATED TO THE FIFTEENTH CENTURY

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The polychrome wooden sculpture is a complex artifact because of the presence of different materials and techniques. Due to changes in style, objects closely related to the cult were often over painted. The present study aim to identify and set-up a correct investigation methodology to identify pigments and binders of the original layer and subsequent overpaintings that often characterize polychrome wooden sculptures, pointing out how the acquisition of these data can be a support not only to the activity of the restorer, who frequently has to face the removal of the overlayers, but also to the deepening of the historical artistic context, connected to the knowledge of the work of art. In this study a multi-analytical approach has been applied on two fifteenth-century Ligurian Christs, the first one from San Bartolomeo parish in Zuccarello, the second from Borghetto San Nicolò, both heavily repainted. The application of different analytical techniques is in fact often successfully for paints analysis [1]. By comparing the results of XRF analysis performed with portable instrumentation on stratigraphic sequences related to samples coming from different color areas of the work, with the data obtained by SEM-EDS, the inert of the different layers could be identified, then confirmed by μ-FTIR that also revealed the chemical nature of the binders employed confirmed and identified by GC-MS analysis [2]. At this purpose both standards and mock up samples prepared following the ancient recipes were analyzed following the conventional approach based on GC-MS analysis and a new methodology based on nLC-MS/MS analysis to characterize in one single shot the protein content. The samples were digested by endoproteases, such as trypsin, and directly analysed by high resolution mass spectrometry. The use of bioinformatic tools resulted in the identification of proteins. The same approach will be used to analyse the real samples [3]. The complete samples analytical characterization has allowed a new possibility of comparison for the classification system of the *" selva dei Cristi feriti liguri quattrocenteschi nel Ponente*", to which the two statues belong, proposed in the text "La Sacra Selva. Scultura lignea in Liguria tra XII e XVI secolo " [4].

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PROJECT "NOPAL": NATURAL COATINGS FOR THE PROTECTION OF LECCE STONE BUILT HERITAGE

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The research project: "*NOPAL: Natural Origin Protective for Artistic buildings in Lecce stone*" sponsored by the University of Salento (*5 per mille per la ricerca-anno 2016)*, aims at developing a new ecofriendly product, that meets the requirements of non-toxicity, biodegradability, and low cost, and able to efficiently preserve the built heritage made of Lecce stone.
In this perspective, the study and development of new materials plays a fundamental role in responding to the growing need of restorers for products with low environmental impact, but which are simultaneously effective and easy to use. In recent years, even in the field of Cultural Heritage, despite the general tendency to cancel almost completely the artistic and artisan tradition of the Salento area, on the other hand there is a return to the origins and the research activities are therefore aimed at experimenting new products starting from those available in nature [1, 2].
Moreover, many fronts of historic buildings (churches and palazzos) in Lecce and the Salento have patinas suggesting the ancient practices of applying natural products.
The work presents the results of the experimentation of products for the protection of heritage built in Lecce stone, made from the cladodium of the species *Opuntia Ficus-Indica*. It is a species belonging to the *Cactaceae* family, naturally available in the Salento area or as a waste derived from industrial processing, whose interest in the scientific community in recent years has increased thanks to its good adhesive, cohesive, water-repellent, and antimicrobial properties.

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SHINING LIKE GOLD AND SOFT LIKE SILK. THE CASE OF BYSSUS SAMPLES COMING FROM THE COMMODITY SCIENCE MUSEUM OF THE UNIVERSITY OF BARI

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The word *byssus* is the zoological term used to call the fine but strong filaments of fibre beard produced by the mollusc *Pinna nobilis*. Byssus was the basic raw material used to make sea-silk, but, given that this species, endemic in the Mediterranean, is protected since 1992 [1], a renewed production of the beautiful iridescent amber-golden textile is essentially impossible. It is proved that the use of sea-silk dates back at least to the Roman age. In the modern times, up to the middle 20th century, Sardinia and Apulia (especially Taranto), were production centers of sea-silk and keep their importance still nowadays in the transmission of the knowledge of the ancient expertise, concerning the procedures of cutting, washing, drying, combing, spinning and -much rarely- waving of the fibre.
The oral handing down of the know-how must be protected as part of our immaterial demo-ethno-anthropological heritage, because it is unfortunately gradually disappearing, like the concept of cultural heritage linked to the precious and rare textile that it produced. Only recently some effort has been made to draw attention on this subject with projects and exhibitions [2].
We studied samples of sea-silk stored at Commodity Science Museum of University of Bari "Aldo Moro" (Fig.1), where one valve, some pearls and all the different phases of crafting of this textile are represented, starting from the raw byssal threads up to the woven textile.

Figure 1. Samples of sea-silk stored at Commodity Science Museum of University of Bari Aldo Moro.

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The aim is to characterize morphologically and chemically the threads, especially the evolution and changing throughout the different working phases. In this way, there will be a scientific trace of what started as an oral tradition and is not yet nowadays widespread and well-known.
We used different techniques like optical microscopy (OM), Scanning Electron Microscopy coupled with Energy Dispersive Spectroscopy (SEM-EDS) and Inductively Coupled-Mass Spectrometry (ICP-MS). The results obtained show how the fiber of the Sea-silk changes until the achievement of the golden "soul of the sea" [3].

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MAPPING APULIAN RED FIGURE POTTERY BY A MULTITECHNIQUE APPROACH

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Apulian red-figured pottery, one of the most important examples of ceramic handcraft production in Magna Graecia, dating back to the 5th and 4th centuries BCE and coming from the most relevant sites in Apulia (Southern Italy), has been extensively characterized by our group for several years [1-5].
Our main goals are various and quite ambitious: highlighting technological differences between Apulian red-figured pottery and the most famous Attic one, obtaining valuable knowledge about pottery workshops and painters and defining the nature of coatings and decorations.
We have investigated ceramic body, black gloss and overpainting areas of items by different techniques according to issues to be solved and samples availability. The ceramic bodies' elemental composition has been investigated by inductively coupled plasma mass spectrometry (ICPMS), the mineralogical composition of pastes by polarized-light optical and electron microscopies (OM and SEM-EDS), and X-ray powder diffraction (PXRD). The fruitful combination of results driven from multivariate statistical treatment of compositional data and mineralogical arrangement of pastes allows us to formulate hypotheses about the provenance of items and manufacturing tradition of workshops, starting to make it possible to comprehend the connections among ceramic technology, artistic expression, and workshop practice in the samples analyzed.
Also, with regard to the material brought to light during the 19th century, it is known that "antiquarian type" of restoration was the most used (i.e. reconstruction and repainting, following the mimetic taste of the time). From this point of view, our archaeometric investigations have also provide detailed guidelines on the 19th century restoration techniques [6].
All 5th century objects analyzed up to know, nevertheless sites of provenance, show the same features: fine texture of the ceramic body, red figures saved from the ceramic paste and black gloss painted directly on the ceramic body. Regarding the 4th century objects, some highlight features similar to the 5th century ones, whereas others are characterized by a ceramic body with a coarser texture and a layer of *ingobbio rosso*. This intermediate layer entirely covered the external part of the vase and was reddish than the ceramic body -visually better to obtain red decorations- and the black gloss -when present- was painted on it.

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The chemical and minero-petrographic results make it possible to discriminate different production technologies of red figured Apulian vases used in Apulia during the 4th century BCE. This technology seems to take shape of a distinctive characteristic of Late Apulian production regardless of sites of provenance.
Finally, we selected a consistent number of items to be analyzed by LA-ICPMS (Laser Ablation ICPMS). We compared Apulian samples and Attic ones, obtaining info on major, minor and trace elements. From an archaeometric point of view, the results showed differences both in the black gloss and in the ceramic body raw materials used in Apulia with respect to Attic ones, so providing an objective parameter of regional production discrimination. The comparison carried on leads us to exclude imports of black gloss from Greece, as hypothesized by some scholars [7]. Nevertheless, in order to accomplish conclusive observations, sampling has to be extended, both in terms of numbers and provenance of samples.

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CHEMICAL INVESTIGATION OF HISTORICAL PASTELS FROM EDVARD MUNCH'S STUDIO

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Edvard Munch (1863-1944) used a wide variety of materials and techniques in his artistic experimentation. The time of Munch's art production was a period of great innovation in chemical organic synthesis and industrial production of paint materials. Hundreds of new appealing artists' materials became available and Munch explored their potentialities in his artworks, without neglecting the use of traditional materials.
The Munch Museum in Oslo (MUM) owns a large collection of Munch's art tools and materials including palettes, brushes, binders, crayons, pencils and paint tubes. In 2014, MUM conservation department started a project dedicated to document and analyse the studio materials to gain new knowledge about materials and techniques, as well as the aging behavior.
This study focuses on the chemical investigation of the MUM's collection of historical oil pastels from the Munch's studio (Figure 1) produced by Lefranc Couleurs and by Dr. Fr. Schoenfeld. Nowadays, there is still an unfamiliarity of the early formulations of oil pastels and crayons produced by different manufacturers in the beginning of the 20th century.

Figure 1. Lefranc Couleurs and by Dr. Fr. Schoenfeld crayons from Edvard Munch's atelier conserved at Munch Museum in Oslo.

We applied a multi-analytical approach based on the application of preliminary non-invasive spectroscopy and micro-destructive mass spectrometric methods.

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The characterization of the organic components constituting the binders and additives was carried out at molecular level by analytical pyrolysis coupled with gas chromatography/mass spectrometry (Py-GC/MS) to evaluate the presence of lipid, proteic or polysaccharide materials. Gas chromatography-mass spectrometry (GC/MS) and liquid chromatography coupled with mass spectrometry (HPLC-ESI-Q-ToF) were applied to achieve both the fatty acids profile and the acylglycerols in the lipid components. HPLC with diode array (DAD) and MS detection complemented the analytical approach to gather information on additives and organic pigments in the formulations. XRF, FTIR, Raman and SERS spectroscopies were also applied, for the identification of the inorganic and organic pigments, fillers and extenders.

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COMPARATIVE CHEMICAL INVESTIGATIONS OF ALUM TREATED ARCHAEOLOGICAL WOOD FROM DIFFERENT MUSEUM COLLECTIONS

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From the mid-1800 to the late 1950s, conservation by alum salts (potassium aluminium sulphate) was a popular method to prevent shrinkage and to impart strength to waterlogged wooden objects, mainly in Scandinavian and Baltic States. The original method consisted in immersing the wood fragments in a hot solution of alum. In 1911 George Rosenberg, modified the formulation by including glycerol. In many cases the objects were coated with various types of oils, such as linseed oil, melted beeswax and shellac or nitrocellulose varnishes after treatment. Today many of the objects treated with alum feature extreme deterioration and very low pH.

In the context of "Saving Oseberg" project we investigated the extent of current chemical degradation in wooden objects conserved with alum salts. We compared samples taken from four different collections: the Dejbjerg collection (1883) at the National Museum of Denmark; the Oseberg collection (1905-13) at the Museum of Cultural History in Oslo; the Glimmingehus collection (1936) at the Swedish History Museum, and objects from the Colonial Williamsburg Foundation, Williamsburg, USA (1950s or 1960s).

The samples were treated using different recipes involving alum salts and other additives, such as linseed oil and/or glycerol. Analyses of lignocellulosic polymers and of inorganic compounds were undertaken. The investigations were performed using a multi analytical approach applying pH measurements, analytical pyrolysis (Py-GC/MS), X-ray diffraction (XRD), scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS) and FTIR.

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SET-UP AND APPLICATION OF AN ANALYTICAL PROTOCOL TO CONTROL IN REAL TIME THE CLEANING OF PAINTED SURFACES OF HISTORICAL AND ARTISTICAL INTEREST THROUGH "SMART" DEVICES

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The Cleaning of painted surfaces of artistical and historical interest is one of the most critical step of every restoration workshop: it is an irreversible and invasive operation that must be carried out maximizing its selectivity. Only the degradation patinas must be removed, minimizing the impact on the materials that constitute the original work of art. To this purpose an optimal cleaning action must be as more as possible gradual and controllable by the conservator [1].
In order to satisfy these requirements, in the last decades, many chemical gels tailored for the cleaning of painted surfaces, have been set up and optimized [2], allowing an improvement of the cleaning performances respect to traditional physical gels.
On the other hand, one of the limits that still exist especially for the cleaning of easel paintings is the absence of a rigorous analytical protocol for the analysis in real time of the materials extracted during the cleaning. Thus, the set-up of a new accurate analytical protocol to monitor the cleaning is mandatory. The attention has been focused on some innovative aqueous Highly Viscous Polymeric Dispersions (HVPDs) composed by polyvinyl alcohol covalently crosslinked by borax, that have been successfully used in the last years for the removal of degradation patinas from the surface of different kinds of works of art [3-5].
The target of this work is the development of a colorimetric assay, starting from previous work applied to natural products [6], for the qualitative and semiquantitative fast monitoring of the materials extracted by the HVPDs taking advantage of common smart devices like mobile phones and tablets as optical readers.
In that way, the cleaning can be tuned on the base of the assays outcomes to prevent any an irreversible modification of the surface.

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HIGHLY SENSITIVE SERS SENSING OF PAINTING DYES THROUGH HIERARCHICAL NANOSTRUCTURES COMPOSED BY SILVER NANOSTARS (AgNSs) DECORATED GOLD NANOSTRUCTURES

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The detection of artistic molecules such as dyes and/or pictorial binders employed in the field of Cultural Heritage requires more and more sensitive techniques. The surface enhanced Raman spectroscopy (SERS) represents a valuable option due to its ability to reveal molecules adsorbed or bound onto nanostructured interfaces. In order to maximize the enhancement of the Raman effect, different Ag and Au nanomaterials where bound together to produce hierarchical structures. In particular, silver nanostars (AgNSs) were chosen because of the strong enhancement caused by their anisotropic shape and they were prepared by one-pot chemical synthesis [1]. The AgNSs were used to decorate two different gold nanostructures, namely Au nanowires (AuNWs) and sphere segment void (SSV) structured surfaces. The AuNWs were obtained in the shape of ensembles of nanoelectrodes by templated electroless deposition in nanoporous membranes [2, 3] while the SSV substrates were prepared via gold electrodeposition by using a monolayer of polystyrene spheres as template [4].
The enhancement of the Raman effect was tested through the employment of different analytes: benzenethiol as Raman probe; cochineal lake was chosen owing to its use throughout the history of art; pure carminic acid as it was the most abundant molecule responsible for the color of cochineal lake.
We compared the magnitude of the enhancements obtained with different SERS substrates: standing alone AgNSs, AuNWs ensembles and SSV substrates and hierarchical nanostructures composed by AgNSs combined to AuNWs (AgNSs@AuNWs) and to SSV substrates (AgNSs@SSV). Particularly strong enhancements were recorded in the case of the AgNSs@SSV substrates, showing the high potential of the so called particle-in-cavity (PIC) architectures [5]. Concluding, we synthesized highly effective hierarchical nanostructures whose future employment lies in the production of extremely sensitive SERS biosensors to be applied in the detection of artistic dyes and binders.

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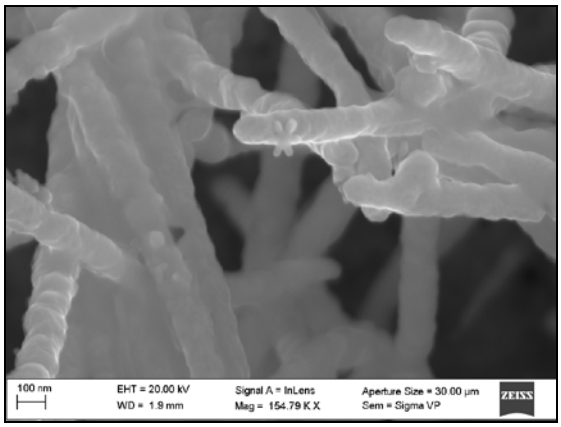
a) 

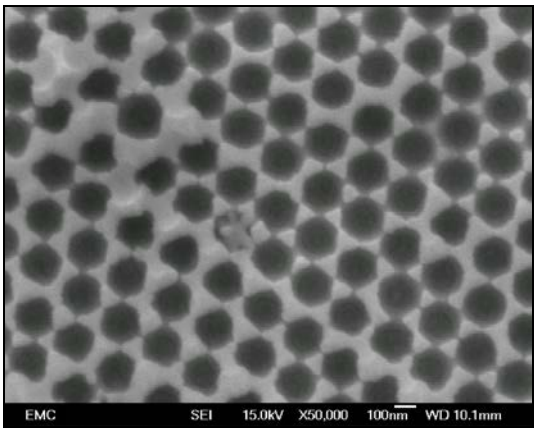
b) 

Figure 1. FEG-SEM pictures of an AgNS a) bound to a AuNWs ensemble and b) hosted inside a cavity of a SSV substrate.

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A NOVEL NON INVASIVE METHOD TO CHARACTERIZE ANCIENT MANUSCRIPTS FROM MIDDLE-EAST

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In the field of cultural heritage the correct conservation of paper artworks plays a fundamental role. In this contest, the determination of paper composition as well as its degradation state is fundamental to determine the suitable restoration and conservation processes for paper artworks. To this end, several diagnostic techniques are available, both invasive and not. This work propose a new "non-invasive" sampling method, its development and optimization, based on the use of sponges, mainly used for cleaning of paper artworks, for the evaluation of the characterization and the conservation status of a group of precious manuscripts of XIII century, collected in the Vatican Library (Biblioteca Apostolica Vaticana – BAV, Vatican City). The paper artworks have been characterized by several techniques using portable instrumentation (colorimetry, X-ray fluorescence (XRF), Infrared Reflectance (IRR), Fourier Transform Infrared Spectroscopy (FT-IR)), directly in the restoration laboratories of BAV. In parallel, the manuscripts are cleaned on specific points using dry cleaning sponges, and the removed materials (pollutants and cellulosic degradation products) have been analyzed by destructive techniques as high pressure chromatography (HPLC) [1], and through no destructive techniques as FT-IR-ATR [2]. Sponges, indeed, absorb dust and degradation products during the cleaning of the paper sheets, avoiding damaging on the paper surface itself. Summarizing, in this work, a non-invasive method of sampling has been developed and optimized based on the analysis of residue materials absorbed by cleaning sponges during the dry cleaning of paper. The validation of the chromatographic results has been performed by non-invasive spectroscopic analysis using a portable instrumentation. The composition and degradation state of these manuscripts have been determined as well as their inks composition. In this work, some of the most significant results are reported.

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P120

PROTONATION OF D-, DL-, L-METHIONINE

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Methionine (2(S)- amino-4-methyl mercapto butanoic acid) is an apolar aminoacid. Its molecule is chiral. It is therefore found in three possible forms: L-, D-, and DL-.Methionine is an aminoacid containing sulfur, because in its molecule is present a methyltioether group.
Methionine in solution can behave as a chelant agent, because it has a carboxylic and an amine function, so that it can form a five membered ring with a cation.
An investigation on the complex formation, involves a preliminary study on the protonation of the ligand. Its protonation was the subject of several studies, but a possible different value of protonation constants of the three chiral species (DL-, D-, and L-) was never considered.
A previous study on the system cobalt (II) – serine [1] showed that the stability constants involving the three chiral forms of serine were a few different each other.
The aim of this work is to verify if the protonation constants of the three forms of methionine are different showing a similar trend above indicated for the serine.
For this purpose, electromotive force (e. m. f.) measurements are carried out at 25°C and in a constant ionic medium, with the following cell:
R.E./ Test Solution / G.E., where G.E. is a glass electrode and R.E. is a reference electrode.
To verify the eventual dependence on the ionic medium, Test solution was prepared alternatively in 1.00 mol dm^{-3} NaCl or 1.00 mol dm^{-3} $NaClO_4$, as ionic media.
From experimental data, i.e.–log *h* (*h* is free concentration of H^+), total concentration of each form of methionine and analytical excess of H^+, the protonation function could be calculated and plotted *versus* –log *h.* The protonation constants of the three chiral forms show a trend similar as above indicated both in NaCl as in $NaClO_4$.

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THERMODYNAMICS OF INTERACTION BETWEEN RISEDRONIC ACID AND METAL CATIONS: INVESTIGATIONS IN SOLUTION AND IN THE SOLID STATE

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Risedronic acid (see Figure 1) belongs to the class of biphosphonates (BP), widely used in the medical field to treat bone disorders. Bisphosphonates (BPs, e.g., alendronate, risedronate, and ibandronate) help to maintain bone mass, to inhibit osteoclast-mediated bone resorption, and to reduce the risk of both vertebral and non-vertebral fractures. The clinical efficacy of BPs is mainly based on two key properties: their capacity to strongly bind hydroxyapatite crystals of bone, and their inhibitory effects on osteoclast precursors and mature osteoclasts. Interaction of risedronate with metal cations is poorly investigated. Qualitatively, it is indicated that adsorption of risedronate is inhibited when the drug is taken with mineral water containing high levels of calcium or magnesium, but chemico-physical results are missing.

In this work, the interaction of risedronate with Ca^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+} is investigated by means of potentiometric and calorimetric measurements to determine thermodynamic parameters (ΔG, ΔH and TΔS). The most important species resulted to be the M_2L and variously protonated MH_iL (with i from 0 to 2) depending on the specific metal cation considered. During all the measurements, the formation of a sparingly soluble species has been noted starting from very acidic pH values (~ 3.5). Thus, four solid samples have been properly prepared and characterized by means of surface spectroscopic techniques (μXRF, XRD, Raman and ATR-IR) and thermal analysis (TG-DTA). In addition, the solubility of these compounds has also been determined by the shake-flask method followed by differential pulse-anodic stripping voltammetry at different NaCl concentrations, to determine Setschenow and activity coefficients. Preliminary results show that the solubility of lead and copper risedronate increases with chloride concentration, probably due to the formation of chloro complexes with Pb^{2+} and Cu^{2+}.

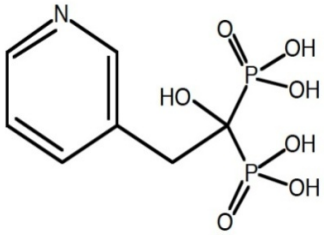

Figure 1. Chemical structure of Risedronic acid

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SYNTHESIS OF A NEW TRIPODAL 3-HYDROXY-4-PYRIDINONE. PROTONATION AND COMPLEX FORMATION STUDIES WITH Fe^{3+}, Al^{3+} AND Zn^{2+}

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Body iron and aluminium overload exerts a number of toxic effects, spanning from tissue damage to organ failure and eventually death. Chelating agents for Fe^{3+} have been used since 1970's to reduce the toxic effects of this metal ion. Despite their efficacy, they also present various drawbacks, so that the research for new iron chelators is always of current interest. Following our recent developments in the study of hexadentate 3-hydroxy-4-pyridinones (3,4-HP) with high sequestering capacity toward trivalent metal ions [1,2], we present here the new tripodal tris-(3,4-HP) (LH_3) below reported. This compound differs from the previous analogues in the corresponding anchoring backbone, which includes one terminal amino group. This amino group, not involved in complexation, is suitable for extra-functionalization, either for improving the lipo-hydrophilic balance, facilitating the crossing of bio-membranes, or for providing sensing or bio-targeting capacity.

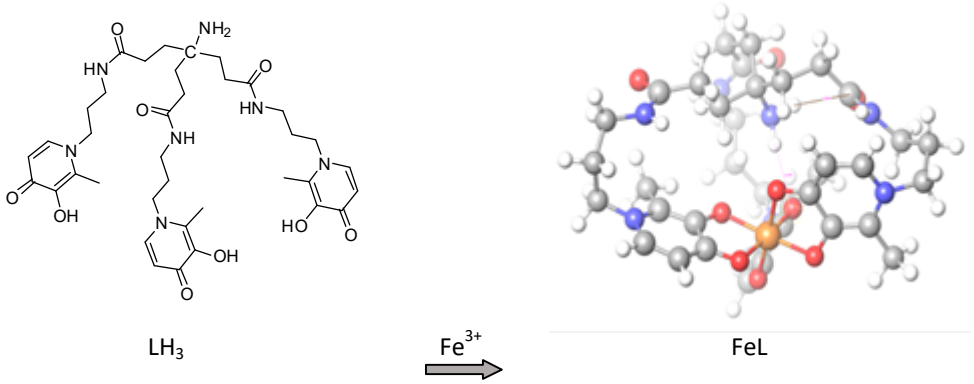

Figure 1. 3,4-HP structure and Fe-(3,4-HP) complex structure

The study of protonation and metal complexation in solution has been performed to evaluate the acid-base properties and complexation capacity towards Fe^{3+}, Al^{3+} using

potentiometry and different spectrometric techniques. The pFe and pAl values present a consistent increase with respect to the similar ligands so far studied. This can be ascribed above all to the high flexibility of the anchoring moiety, and to the adequate length size of the arms connecting both moieties, as also indicated by the DFT model of the FeL complex.
The complexation capacity of this ligand with Zn^{2+} was also evaluated, and it was proved that the strong sequestration capacity towards the target trivalent metal ions does not lead to depletion of this essential metal ion.
The capacity of the new ligand to facilitate metal-mobilization from the body has been also studied using a mice model injected with ^{67}Ga, showing extremely interesting ability to remove iron and aluminium ions under *in vivo* conditions.

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BINDING ABILITY OF SOME AMINOACIDS AND NUCLEOTIDES TOWARDS As(III): A COMPARISON

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The increase of heavy metal concentration in the environment, especially for anthropogenic reasons, is source of serious concern regarding human health and global ecosystems. As it is well known, the toxicity, bioavailability and mobility of a metal are linked to the physico-chemical form in which the element is present in an existing sample and, thus, a speciation analysis is required to better evaluate all these phenomena [1]. As regards arsenic, in fact, it can be found in both organic and inorganic forms. Among these ones, the inorganic species are the most harmful and, in particular, arsenic in its trivalent oxidation state, since it shows the capability to interact with the sulfhydryl residues of the proteins, hindering their biological functions [2].Unfortunately, this metalloid can reach human organism through many pathways but it seems that the main source of arsenic intake is to be found in the consumption of contaminated water and food [3].
In order to investigate the behavior of As(III) in presence of biological molecules, a thermodynamic study was performed in aqueous solution by using some aminoacids and nucleotides as ligands. The aim is also to enrich the existent literature, since to our knowledge, no data are reported. The compounds under investigation are listed in Table 1. For each one, the speciation model was determined by means of potentiometry at I = 0.15 mol L^{-1} and t = 25 °C.

Table 1. Ligands under investigation

Ligands	Acronym
Glycine	*Gly*
L-Aspartic acid	*Asp*
Lysine	*Lys*
Adenosine 5'-monophosphate sodium salt	*AMP*
Adenosine 5'-diphosphate sodium salt	*ADP*
Adenosine 5'-triphosphate disodium salt hydrate	*ATP*

In order to analyse the binding ability of all the ligands towards the metal, the $pL_{0.5}$, an empirical parameter already proposed by the research group, was employed [4]. It represents the total ligand concentration required to sequester the 50% of a metal present

in traces and it takes into account all the variables that could affect the estimation of the complexing capability, such as the acid-base properties of the ligand, the hydrolysis of the metal and all the potential interactions with other components in solution. It is represented by plotting the mole fraction of the complex species *vs.* pL, where pL = -log C_L (C_L = total ligand concentration). Graphically, it is a sigmoid curve with asymptote 1 for pL → - ∞ and 0 for pL → + ∞.

$$\chi = \frac{1}{1 + 10^{(pL - pL_{0.5})}}$$

The study of the sequestering ability was performed at *I* = 0.15 mol L^{-1}, t = 25 °C and at various pH values, in order to establish in which conditions the ligands better interact with As(III).

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P124

BEHAVIOR OF GLUTATHIONE AS LEAD-LIGAND

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The presence and mobilization of heavy metal cations represents under many aspects a current and important problem in the environmental field. In this research, as cation lead (II) ion was studied. On the other hand, glutathione (GSH), present in plants, is potentially able to form complexes with cations, in this case with lead (II), so that it could be useful for a possible improvement of environmental remediation technique (phytoremediation). The formation of complexes between glutathione (below indicated with *L* in its completely deprotonated form) and lead (II) was studied at 25° C and in 1.00 mol dm^{-3} NaCl as ionic medium by means of measurements of electromotive force (e. m f.) of the following galvanic cells:

Pb (Hg)/Solution Test/ R.E. and R.E./ Solution Test/ G.E.

where Pb (Hg), and G.E. are amalgam of lead and glass electrodes respectively, whereas R.E. is the reference electrode. Beside to the e. m. f. measurements, spectrophotometric and N.M.R. measures were carried out to obtain information on the type of links existing between *L* and Pb (II). Previously, it was necessary to study the protolytic behavior of glutathione in the same experimental conditions of temperature and ionic medium. The experimental data, obtained from e.m.f. measurements performed with a glass electrode, are well explained assuming the following species:

$H_4 L^+$, $H_3 L$, $H_2 L^-$, HL^{2-}

The relative protonation constants defined as:

$$[H_n L] = h\, K_n\, [H_{n-1} L]$$

where square parentheses indicate the free concentrations of the inside species and *h* is the free concentration of hydrogen ion. The following values are obtained:

$\log K_1 = 9.55 \pm 0.03$, $\log K_2 = 8.46 \pm 0.02$, $\log K_3 = 3.45 \pm 0.05$, $\log K_4 = 2.25 \pm 0.10$.

The e. m. f. experimental data obtained in a wide range of both analytical excess of concentration hydrogen ions, and -log *h*, and of total concentration of lead (II) and of glutathione were explained by assuming the presence of mono-nuclear complexes in lead (II), but with the participation of the hydrogen ion. The formation of Pb H *L*, Pb H_2 *L* and Pb H_3 *L* (omitted charges), with the relative stability constants, explains the experimental data very well. Spectrophotometric measurements carried out with the Job method confirm the presence of complexes in the ratio 1: 1 between cation and ligand. The N.M.R. data show the formation of a chelate between Pb (II) and carboxyl and sulfur present in the glutathione molecule.

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FOLIC ACID PROTONATION AND SOLUBILITY

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The folic acid (HFol) or pteroil (mono) glutamic acid or vitamin M or vitamin B9 is necessary for all the reaction of synthesis or reparation or metylation of DNA, for omocystein metabolism and for other biochemical reaction. It is constituted by three molecules: 6-metilptherinae, p-aminobenzoic acid and glutamic acid.
Few studies are present in the literature [1, 2] and disagreement data relative to its protonation constants and solubility are shown.
The researches of Poe [3], Szakács and Noszál [4] are relevant because they showed the very low solubility of HFol and several protonation constants. However, the obtained values are determined at different ionic strength and not in agreement each other.
The aim of our research is to determinate accurately the solubility of HFol and all its protonation constants in 1.00 and 0.15 mol dm^{-3} constant ionic media so that it is possible minimize the variation of activity coefficient reagents [5]. Furthermore, the position of the protons on the structure of HFol is investigated by NMR measurements.
Different types of measurements are carried out.
Solubility (*S*) is investigated, at 25°C in constant ionic medium, putting in contact solutions at different values and in a large range of *H* (analytical excess of hydrogen ion) with an excess of HFol. After 24 hours the solutions are in equilibrium and by centrifugation and filtration through Millipore filters, the –log *h* (free concentration of H^+) and the absorbance are measured. From this approach, it is evident a plot log *S versus* –log *h* shows the minimum value corresponding to the solubility of HFol (*s*) and a further protonation of HFol.
Electromotive force (e. m. f.) measurements are performed at 25°C and in constant ionic media (1.00 and 0.15 mol dm^{-3} NaCl) of the galvanic cell: R.E. / Solution Test/ G.E., where R.E. and G.E. are reference and glass electrode respectively. The test solution contains known *H* and an excess of HFol in order to have constant its activity. The test solution is titrated with a basic solution in constant ionic media and at known concentration.
Other (e. m. f.) measurements are performed in the same experimental conditions, but in alkaline solution where Fol in the completely deprotonated form is present. An acid solution in constant ionic media is added until the formation of the first cloudiness.
From both (e. m. f.) measurements the protonation function can be obtained and by using the obtained solubility datum the protonation constants can be obtained.
NMR and CD measurements are performed to have information on the positions of the protons on the stricture of folic acid. Particular interest is dedicated to the further proton assumed by HFol at $-\log h \leq 3$.

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INVESTIGATION ON THE THERMODYNAMIC PROPERTIES OF TWO ANTIBACTERIAL DRUGS IN AQUEOUS SOLUTION

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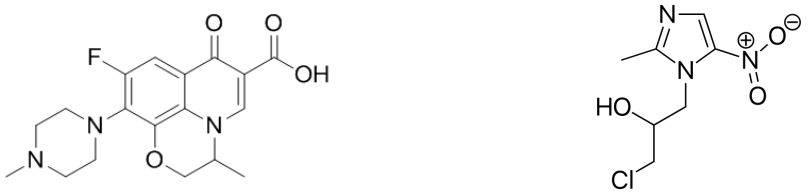
This contribution is the result of a speciation study of two ligands, namely ofloxacin and ornidazole (Figure 1), in NaCl aqueous solution and at different experimental conditions.



a) b)

Figure 1. Molecular structures of ofloxacin (a) and ornidazole (b)

They are synthetic antibacterial drugs commonly used for the treatment of urinary tract and intestinal protozoan infections, respectively. Ofloxacin is a fluoroquinolone and belongs to the class of quinolones, while ornidazole is a derivative of 5-nitroimidazole and has a significant efficacy on patients, due to its long half-life time. The biological relevance of these ligands led the research group to study their speciation, which indicates the distribution of the different physical and chemical forms in which a component is present in a system. Since these various forms may have different behavior towards humans and environment, the speciation study becomes important to have information about their bioavailability, toxicity and environmental impact. [1-2] The acid-base properties and solubility of ofloxacin and ornidazole were investigated by means of potentiometric and UV-Vis spectrophotometric measurements performed at $0.15 < I/\text{mol L}^{-1} < 1.00$ and $15 < t/°\text{C} < 45$. NaCl was chosen as ionic medium, since it is the principal inorganic component of many biological fluids. [3] The elaboration of experimental data allowed to determine two protonation constants for ofloxacin, likely attributable to the nitrogen atom in position 4 on the piperazine ring and to the -COOH group, and one for onidazole, probably due to the –OH group. From the data obtained at different temperatures, protonation enthalpies were calculated using the Vant'Hoff equation, highlighting a moderate dependence of the protonation constants on this variable. Solubility measurements performed on small aliquots of ligands, led to the determination of the total and neutral species solubility value at various experimental conditions and to the calculation of dissolution enthalpies at $I = 0.15$ mol L^{-1}.

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ON 4-METHYL-7-(2-PYRAZINIL)-2H-[1,2,4]TRIAZOLO[3,2-c][1,2,4]TRIAZOLE AQUEOUS SOLUTIONS

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Heterocycles are compounds among the most important of synthetic chemistry and have important application as drugs and bioactive compounds. The aromatic heterocycles play a fundamental role in many emerging fields of organic electronics and optoelectronics: conductive polymers, organic field-effect transistors [1], organic solar cells [2], optically active nonlinear compounds [3,4]. Studies of the structural, tautomeric and acid base properties of [1,2,4]triazolo[3,2-c][1,2,4]triazole system, (Fig. 1), a 10-electron aromatic N-rich fused heterocycle, characterized by the presence of substituents of different electronic character (electron donor or acceptor) onto the bicycle, have been conducted and described in the literature [5,6].

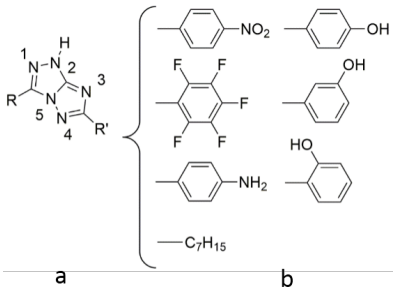

Figure 1. a) Chemical diagram of [1,2,4]triazolo[3,2-c][1,2,4]triazole, with atom numbering, in which only the 2*H* tautomer is shown; (b) Chemical diagrams of triazole compounds studied in the previous works.

In this work we propose a thermodynamic study on the 4-Methyl-7-(2-pyrazinil)-2H-[1,2,4]triazolo[3,2-c][1,2,4]triazole aqueous solutions at 25°C, in 0.5 M NaCl. Acid-base, redox and complexation properties (with Cu^{2+} and Zn^{2+}) have been investigated by potentiometry, spectrophotometry (absorption and emission) and voltammetry.

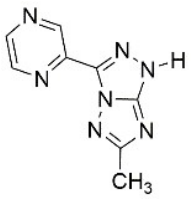

Figure 2. 4-Methyl-7-(2-pyrazinil)-2H-[1,2,4]triazolo[3,2-c][1,2,4]triazole

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The experiments have been performed as acid-base titrations at constant concentration of the triazolo compound, without varying the 0.5 M level of [Cl-]. The pH investigated spans between 0.3 and 13. The equilibrium free proton concentration was evaluated by measuring the electromotive force at the ends of a galvanic cell (I)

G.E./T.S./R.E. **(I)**

where T.S. indicates the Test Solution, G.E. is the glass electrode and R.E. is a reference electrode. The voltammetric measurements have been conducted with hanging mercury drop electrode (HMDE or HDME) as working electrode and Ag/AgCl/KCl 3 mol*dm^{-3} (Reference electrode). In Fig.3 UV-VIS spectra (absorption) at different pH values are reported: increasing pH, a bathochromic shift is observed.

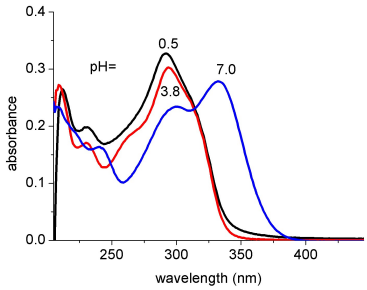

Figure 3. Absorption spectra of 4-Methyl-7-(2-pyrazinil)-2H-[1,2,4]triazolo[3,2-c][1,2,4]triazole 3·10^{-5} M solutions, in 0.5 mol/dm^3 NaCl-4% ethanol, at various pH values.

The primary spectrophotometric data, A^{λ}/pH, and, I^{λ}/pH, were numerically interpreted by the HYPERQUAD program [6].

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A NOVEL PROCEDURE FOR THE FAST IRON SPECIATION IN SEAWATER

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Iron determination and speciation in seawater has attracted considerable attention in chemical oceanography since early findings about iron limitation. It is now recognized that very low, subnanomolar, iron concentrations limit primary productivity, i.e. photosynthetic activity, in vast areas of the oceans: the relevance of such limitation for CO_2 sequestration from the atmosphere and, more generally, global climate has also been discussed [1–3]. Apart from climate implications, iron plays a major role in oceanic biogeochemical cycles [3]. A comprehensive picture of its biogeochemical cycle in seawater and connections to other global cycles would accordingly represent a giant step forward in our understanding of regulatory processes in the oceans. Understanding the different forms of iron (redox, inorganic, organically bound, colloidal, etc.) is a further step forward in the Speciation analysis fundamental, e.g., to try and define the bioavailable iron fraction [4–6]. From the analytical chemist's point of view, the speciation analysis of iron in seawater at the ultratrace level is a challenging task, requiring the detection of different forms of iron at an iron total concentration usually below 1 nM. Extreme detection capabilities and selectivity are accordingly required.

Aim of this discussion is to present a novel Competitive Ligand Equilibration-Cathodic Stripping Voltammetry (CLE-CSV) method [7] for the speciation analysis of iron in seawater. This method allows the determination of the concentration of complexing organic ligands and their stability constant in seawater.

A recently introduced [8] instrumental configuration was applied for the first time for the speciation analysis, which features a 1 mL microcell and a silver wire pseudoreference, enabling the tenfold reduction of the sample volume. 2,3- dihydroxynaphthalene was used as the competing ligand for iron and atmospheric oxygen as the catalytic enhancer because they ensure the best analytical performances in terms of detection capabilities. The method was optimized and validated using ligands with known stability constant for iron in UV digested seawater. The resulted ligand concentrations and their stability constants with iron were not statistically different from the expected values.

The method was lastly applied to under pack seawater samples from Antarctica giving ligand concentrations between 1.24 nM and 3.17 nM and an average $\log K'_{FeL}$ of 20.15. The experiments confirmed that the novel method can be applied to the speciation analysis showing a great improvement of the analytical performances. Compared with the other methods employing DHN, a tenfold reduction of the sample together with a tenfold improvement of the sensitivity were achieved. Moreover, the enhanced sensitivity enabled an overall 2/3 reduction of the analysis time.

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LABEL-FREE SORTING OF A MULTIPOTENT CELL POPULATION OF HUMAN AMNIOTIC FLUID STEM CELLS: DIFFERENCES IN THE TRANSCRIPTOME

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Amniotic fluid has been used for decades as diagnostic tool to identify chromosomal aberrations or mutations in the foetus. This fluid is an interesting source for cell therapy because it contains stem cells originated from the foetus that can be isolated and stored for future cell therapy approaches (Human Amniotic Fluid Stem Cells, hAFSCs). These cells are very heterogeneous, deriving from different foetal organs such as urinary, gastrointestinal system and skin. Antibody sorting for the stem cell factor CD117 can be used to isolate a highly multipotent subpopulation. Even if only 1% of total amniotic fluid cells has been shown to be CD117+, a strong heterogeneity continues to be shown inside the population [1]. Therefore, new sorting approaches are investigated in order to make cell populations uniform and to have a quality control step for the clinical use of these cells. Within the same heterogeneous cell population, cells can differ in terms of morphology and intracellular composition. Celector® (Stem Sel ltd.) is a new technology that exploits the Non-Equilibrium Earth Gravity Assisted Field Flow Fractionation principle (NEEGA-FFF) to characterize and sub-fractionate hAFSCs based on their solely physical characteristics such as dimension, density, morphology and rigidity. Cells are injected into a capillary device in a sterile condition and they are separated in a label-free mode. At the capillary device outlet, a camera is placed to record the eluting cells and an imaging software generates a specific profile (cells vs time). Generally bigger/denser cells elute earlier then smaller ones and can be collected and use for further studies or direct applications [2].
Amniotic fluid stem cells were derived from discarded cultured cells from 5 clinical routine amniocentesis from mid gestational age patients (16-17 weeks). Cells were sorted for the stem cells factor receptor CD117 and then expanded in culture. Once a sufficient cell number was reached, approximately 2 million of cells, cells were then analysed by Celector®. 300,000 cells were processes per analysis at a flow rate of 1 ml/min. Based on profile and cell live imaging, the population was divided in 4 fractions. Consecutive analyses were run in

order to obtain a sufficient number of cells per fraction. Fraction 1 and 2 (F1-F2) were composed of cell aggregates and the largest cells, while cells from fraction 3 and 4 (F3-F4) were smaller and with sharp edges. Once collected, cells from each fraction were centrifuged and pellet was frozen for RNA extraction. RNA-seq analysis (High Output Illumina Nextseq) was performed for each fraction obtained. Output data were analysed for Gene Set Enrichment Analysis (GSEA) using different public datasets (Hallmark, KEEG and Reactome) and 2 experimental lists (IttaiBP et al., Nature genetics, 2008).
Differential analysis on output data showed an overall change among fractions with significant differential expressed pathways, in particular an upregulation of five pathways (Stemness, DNA repair, E2F targets, G2M checkpoint and Hypoxia) and a downregulation of 4 pathways (EM transition, Mtorc1 signalling, Unfold Protein Response, P53 signalling) in the late eluted cells (F3-F4) compared to F1 and F2 fractions. These results revealed a different preservation of stemness properties in the fractions.
In conclusion, the separative technology Celector® is able to enrich the multipotent component from heterogeneous population of hAFSCs, without extra manipulation. This enriched population of cells can be used for several applications in basic and applied research. These results show an interesting technology to obtain homogeneous and "good" cell therapy products to improve the success of regenerative medicine applications.

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SILVER NANOPARTICLES PLASMONIC SENSOR FOR THE DETECTION OF MERCURY IONS (Hg^{2+}) IN AQUEOUS MEDIUM

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Metal nanoparticles exhibit Localized Surface Plasmon Resonance (LSPR) and this property is widely used for chemical and biological sensing. The colour of nanoparticles is strictly connected with LSPR and depends on shape, dimensions, capping agent and refractive index of the medium in which nanoparticles are dispersed. In this sense a change in LSPR absorption correspond to a colour change of nanoparticles solutions and this feature can be exploit for colorimetric sensors. [1,2]
In this study we report a simple method for synthetize different types of silver nanoparticles (AgNPs) that were largely characterized by UV-Vis spectroscopy, Scanning Electron Microscopy (SEM) and Dynamic Light Scattering (DLS).
Sizes and shapes of AgNPs were controlled by using PVP, Sodium Citrate and $NaBH_4$ as reducing agent, by varying the concentrations of H_2O_2 showing changes in colour of nanoparticles solutions. The colours of nanoparticles varied from yellow to blue and finally to green. From SEM analysis the different colour was associated to different shape that varied from nanospheres, nanoprism, nanorods, and hexagons.
In particular, blue AgNPs were used as sensor for different concentration of Mercury ion (Hg^{2+}).
In fact, these nanoparticles were able to discriminate Hg^{2+} from other metals in the aqueous solution with high selectivity demonstrated by specific LSPR blue shift from 620 nm where a change of 1 nm corresponded to 0.029 nM of Hg^{2+}.

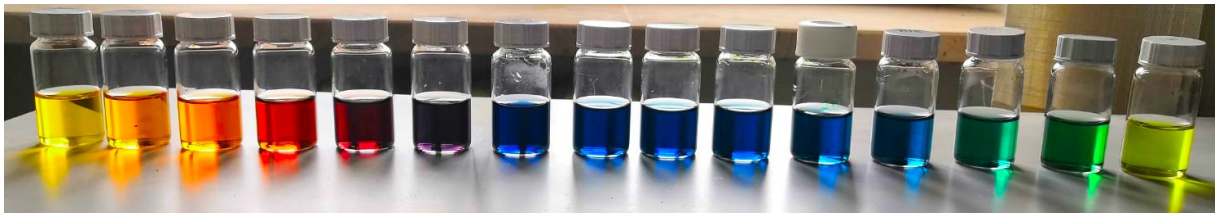

Figure 1. Silver Nanoparticles

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TRACE ELEMENTS IN WILD AND FARMED ATLANTIC BLUEFIN TUNA (*THUNNUS THYNNUS L.*) FILLET

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Environmental pollution by metals is a recognized problem worldwide and it has been demonstrated that some marine organisms may bioaccumulate metals in their tissues [1]. Tunas are an important source of nutritional molecules and elements, but, being at the top of trophic chains, it can accumulate conservative contaminants such as heavy metals [2]. Consequently, from an ecological and safety point of view it is important to know the content of these pollutants in this specie, widely consumed as food.
In this study, trace elements (Cd, Pb and Fe) levels were determined by atomic absorption spectrometry in the muscle of Mediterranean bluefin tuna (*Thunnus thynnus*). A total of 68 samples were collected: wild samples (*n* = 30) were captured near Sardinia island (Italy) and farmed samples (*n* = 38) were from *Fish and Fish Ltd*. fish farm (Malta). Mean trace elements content (mg kg^{-1} wet weight) in wild and farmed, respectively was found as: Cd, 0.014 and 0.021; Pb, 0.11 and 0.026; Fe, 13 and 7.5. Concerning priority pollutants (cadmium and lead) all samples showed values well below the EU limit. A comparison between the two groups showed that no statistically significative difference was found for Cd whereas for Pb wild samples had concentrations significantly higher (about 4 fold) than the farmed ones ($p = 1{,}97 \cdot 10^{-8}$). The level of Fe demonstrated that both groups could be considered good products for the intake of this element, although lower levels were found in the farmed group. Relations between metal concentrations and biometric parameters were evaluated: generally, no correlation with weight were found, whereas some correlation with size were highlighted. Tuna food safety was evaluated considering the European maximum levels for contaminants in foodstuffs (EU 1881/2006): for Cd and Pb both groups represent a safe food, even if farmed specimens are safer concerning lead.
Concerning Fe the recommended dietary allowance (RDA) is 14 mg/day; therefore, both tuna groups supply about 15% of Fe content daily providing a good quote of this essential element.
From these results we can conclude that farmed tuna, such as the wild one, represents a safety and healthy seafood for the consumers, encouraging the farming activities due to an ever growing consuming of this fish.

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