Comunicazioni orali

LIGHT-ACTIVATED ELECTROSYNTHESIS OF MICROSTRUCTURED MOLECULARLY IMPRINTED POLYMERS FOR SENSING APPLICATIONS

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In the past few years, micro- and nano-structured molecularly imprinted polymers (MIPs) with excellent sensing properties, as shorter response time, and simpler detection schemes with respect to bulk films, have been fabricated (1-2). Very recently, miniaturized MIPs have been prepared by electropolymerization leading to the design of MIP nanoparticles (3), microrods (4) and nanowires (5). Nevertheless, a restricted variety of achievable microstructures has been explored so far. The present work describes an original approach for MIP microstructuring based on light-activated MIP electropolymerization on microstructured n-type silicon (n-Si) substrates prepared by electrochemical micromachining (6). Microstructured MIP morphologies can be fabricated with high flexibility by tuning features at the microscale of the microstructured silicon substrates. Imprinted PPy for amino acids is electrosynthesized on different microstructured Si, e.g. featuring an array of square-like pores with size of 5 μm and depth from 5 to 50 μm. Microstructured MIPs are deposited galvanostatically under back-side illumination of n-Si, eventually removing the template by subsequent PPy overoxidation. MIPs are analyzed by Scanning Electron Microscopy (SEM) and their rebinding ability is electrochemically and optically tested. SEM analysis highlights a uniform MIP deposition perfectly replicating the micromachined silicon features. The imprinting effect is verified by comparing MIP and not imprinted films electrochemical and optical responses. MIP selectivity is evidenced by checking its ability in rejecting closely related compounds. The key role of micrometer-scale morphology in enhancing MIP recognition properties emerges from the comparison with flat MIP performances.

The proposed novel approach for the design of MIP microstructures conjugates the flexibility of electrochemical micromachining techniques with electrochemical imprinting technology, thus leading to the development of novel MIP films whose features at the microscale can be controlled and tailored to the specific applications.

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DEVELOPMENT OF SENSORS BASED ON SCREEN PRINTED ELECTRODES MODIFIED WITH CARBON BLACK AND GOLD NANOPARTICLES NANO-COMPOSITE

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In the last years a wide variety of electrode systems, especially based on nanostructured materials was explored, aiming at improving the analytical performance of electrochemical sensors in terms of sensitivity, selectivity, stability, etc. Recently the group of Tor Vergata developed electrochemical sensors based on screen printed electrodes (SPEs) modified with carbon black (CB). The CB-SPEs showed an enhanced oxidation current for several analytes when compared with bare SPEs (1-3). At the same time, the group in Modena has acquired wide expertise in the use of gold nanoparticles (AuNPs) in electroanalysis: effective electrocatalytic processes have been activated by fixing AuNPs on electrode surfaces (4,5).
In this work SPEs were modified by depositions consisting of CB and AuNP nano-composites (AuNPs-CB-SPEs). Using a fixed amount of CB to modify the SPEs (1), the amount of AuNPs was optimized. The AuNPs-CB-SPEs were characterized by morphological and electrochemical techniques, demonstrating that *i)* the CB coating was very stable fixing AuNPs; *ii)* the AuNPs fixed on the CB lead to a nanostructured, very homogeneous composite; *iii)* the nano-composite material is characterized by an enlarged electroactive surface with respect to both the single modified components. Several analytes, such as NADH and glutathione, have been tested on AuNPs-CB-SPEs, demonstrating an improvement of electrocatalytic properties when compared with SPE modified either with CB or AuNPs as the single component. A synergic effect of the two materials is evidenced. The sensor modified with AuNPs-CB nano-composite was also tested for As (III) detection, using anodic stripping voltammetry. The different experimental conditions were investigated and optimized, allowing the detection of As(III) at the legal limit level in drinking water (10 ppb).

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AN ELECTROCHEMICAL GAS SENSOR BASED ON PAPER SUPPORTED ROOM TEMPERATURE IONIC LIQUIDS INTENDED FOR THE ANALYSIS OF ACID SPECIES

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The gas sensor here described consists of three electrodes screen printed with carbon ink onto a filter paper foil where a miniaturized three electrode cell is defined by a circle of hydrophobic wax barrier (1). The back face of this cell is insulated by thermally laminating a polyethylene layer to prevent electrolyte leakage and gas permeation during analysis. A controlled volume (1,7 μL) of a room temperature ionic liquid (RTIL) mixture (2% v/v 1-butyl-3-methylimidazolium acetate [BMIM][Ac] and 98% v/v of 1-butyl- 3-methylimidazolium bis(trifluorosulphonil)imide [BMIM][NTF$_2$]) was then laid on a corner of the paper device in order to soak in paper channels, without covering the upper surface of electrodes. Highly sensitive responses are provided by this membrane free gas sensor thanks to a careful control of the RTIL amount wicked on the paper and of the screen printing of electrodes which permits an intimate contact between RTIL and electrode material at the probe surface to be achieved, so as to allow analytes to undergo charge transfer as soon as they reach the resulting interphase. Thus, fairly slow steps such as analyte diffusion or dissolution in a conductive medium are avoided. The advantage offered by the addition of small amounts of [BMIM][Ac] to the [BMIM][NTF$_2$] electrolyte consists in the appreciable shift towards less positive potentials caused on the oxidation process of acid gaseous species, thus avoiding that they occur quite close to the electrolyte discharge. Such a profitable effect has been exploited for achieving very good performance in the flow injection analysis of phenol and 1-butanethiol chosen as model electroactive gaseous analytes.

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AFFINITY ELECTROCHEMICAL BIOSENSORS BASED ON NANOELECTRODE ENSEMBLES

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Nanoelectrode ensembles (NEEs) are prepared by template deposition of metal nanoelements within the pores of microporous polycarbonate track-etched membranes (1). These devices show remarkable advantages in comparison with conventional electrodes thanks to their particular geometry, such as highly improved signal-to-background currents ratio and detection limits that are 2-3 orders of magnitude lower (1-3).

Functionalization of NEEs with biorecognition elements allows one to fabricate electrochemical biosensors useful for protein or DNA detection. Recently, we introduced an approach for the modification of the NEEs by immobilizing antibodies onto the wide polycarbonate surface surrounding the nanoelectrodes (4, 5), that constitutes the majority of the geometric area of a NEE. The immunosensor was used for the detection of the receptor protein HER2

In this communication, we report the use of NEEs as suitable platform for the fabrication of DNA biosensors where either, the template membrane which surrounds the nanoelectrodes or the nanoelectrodes themselves, are exploited for the immobilization of the biorecognition macromolecules. For the functionalization of the polymer it is important to exploit its natural reactivity with respect to the probe DNA sequences or, eventually, to increase such a reactivity with suitable activation procedures (6).

In the case of the functionalization of the gold nanodisks, the very small metal surface can be a limit for the immobilization of DNA strands. For this reason, the possibility to increase the active area of a NEE by structures provided with high surface area has been studied. In particular, preliminary applications of these 3D-NEEs as biosensors have been faced.

Advantages and limits of these two approaches are compared and discussed.

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RATIONALIZATION OF THE SIGNAL DRIFT NATURE OF OXYGEN OPTICAL SENSORS AND ITS EXPERIMENTAL CHECK WITH A LIGHT INTENSITY DETECTION BASED SENSOR

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A way to quantify the signal drift causes of an oxygen optical sensor has been developed. Theoretical formalization were experimentally confirmed by using a polysulfone-based thin layer membrane embedding platinum meso-tetra-(pentafluorophenyl)-porphyrine working in light emission detection mode. Photochemical, thermal and oxidative degradation of both luminophore and polymeric matrix were rationalized and tested. Experimentally determinable light intensity drift, D_I, and Stern-Volmer constant (K'_{SV}) drift, D_K, were related to matrix and luminophore modifications. The mathematical modeling of the drift evidenced that D_I, is constant by increasing the $\%O_2$ only in the absence of D_K. It has been possible to quantify all the contributions independently through a very good match between theory and experiments. The sensor drift analysis allowed understanding whether luminophore and/or polymer degradations were operative. In the studied case oxidative degradation was absent; thermal degradation of the sole luminophore, was independent of $\%O_2$ and caused a relative light intensity drift of $-0.028(0.002)\ day^{-1}$; photochemical degradation was present both on luminophore and polymeric matrix. When $D_K \neq 0$, also the commercially available oxygen optical sensors based on phase-shift and the life-time detection modes lead to incorrect oxygen concentration values and should account for the drift analysis proposed in the present study.

DETERMINATION OF PCB IN SOIL SAMPLES USING MICROWAVE ASSISTED EXTRACTION FOLLOWED BY GAS CHROMATOGRAPHY

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Polychlorinated biphenyls (PCBs) are a group of persistent organic pollutants that consist of different congeners having biphenyl as the core structural unit with a variable number of Cl-substituents. Basically, PCBs were extensively industrially utilized in open (as additives to glues, dyes, and construction materials) and in closed systems (coolants and lubricants in transformers, dielectric fluids, hydraulic fluids) (1). PCB toxicity is well documented (2). PCBs are resistant to degradation, have long persistency, accumulate in environmental compartments and can enter human body *via* inhalation, direct contact and food chain. They can be transferred across the placenta and into maternal milk (3) and in 2001 were included in the global Stockolm Convention on POPs. PCB are considered substances subjected to review for possible identification as priority substances or priority hazardous substances according to the directive 2008/105/EC. Seven PCBs (Arochlor 1242, 1254, 1221, 1232, 1248, 1260 and 1016) were placed on the list of priority contaminants under the EPA Clean Water Act.

The aim of this study was the optimization of an extraction procedure, using innovative microwave equipments, with high recovery yields, easily routinable for the GC-ECD determination of PCBs in soil.

Certified reference materials of Aroclor 1260, Aroclor 1254 and Aroclor 1242 in transformer oils were used to contaminate the soil samples and to optimize the method. The study was performed optimizing: (i) the extraction; (ii) the purification and (iii) the gas chromatographic separation conditions. After optimization, the recovery yields were included within the range 79-84%. The DL, evaluated for two different commercial PCB mixtures (Aroclor 1260 and Aroclor 1242) were 0.056±0.001 mg/kg and 0.290±0.006 mg/kg, respectively.

The method, validated with certified soil samples, was used to analyze a soil sample after an event of failure of a pole-mounted transformer which caused the dumping of PCB contaminated oil in soil.

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DEVELOPMENT AND OPTIMISATION OF AN HPLC/MS/MS METHOD FOR THE DETERMINATION OF PHENOLIC ACIDS AND DERIVATIVES USING A RP-AMIDE STATIONARY PHASE

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A method for the HPLC-MS/MS analysis of phenols, including phenolic acids, with an amide-embedded phase column (Ascentis Express RP Amide, Supelco) was developed and compared with the ones using classical C18 stationary phase columns.

RP-Amide is a new generation of polar embedded stationary phase, whose wetting properties allow us to work with an high percentage of water as eluent, up to 100 % (1, 2). The increased retention and selectivity for polar compounds and the possibility of working in 100 % water conditions make this column particularly interesting for the HPLC analysis of phenols.

Chromatographic separation was optimised allowing us to obtain the separation of 12 standard phenols. The ionisation condition and the acquisition parameters through Q-ToF detector were optimised; in particular the acquisition was performed in negative polarity and MS/MS target mode. In particular, the optimization of MS/MS detection for each analyte was carried out by working on energy collision and fragmentor potential. The performance of the method was evaluated on the basis of different parameters: linearity, sensitivity, precision, accuracy and repeatability.

The optimized procedure was successfully applied to determine the phenolic content in samples from complex matrixes such as tannin dyestuffs and wood extractives.

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MULTI-WALLED CARBON NANOTUBES-MODIFIED SILICA MICROSPHERES: A NEW HPLC STATIONARY PHASE

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Designing stationary phases based on the excellent adsorption properties of nanomaterials is a hard but fascinating challenge; in this context, the use of carbon nanotubes (CNTs) for preparing novel chromatographic sorbents is a current trend in analytical chemistry (1).
In the last years we have synthesized chemically-modified CNTs, which gave excellent performance as GC stationary phases, behaving as mixed-mode separation materials (2-4).
In this contribute we present the first results on the application as HPLC stationary phase of a novel material based on silica microspheres functionalized with multi-walled CNTs (MWCNTs). These were grafted by γ radiation onto silica microspheres in presence of polybutadiene (PB) as the linking agent. The final product (MWCNT-PB-modified silica) showed homogeneous particle size and high surface area. The chromatographic application of MWCNT-PB-modified silica gave interesting results in the separation of different classes of compounds: aromatic hydrocarbons, chloroaromatics, and a variety of substituted benzenes. Satisfactory resolution and selectivity were obtained also for closely related analytes, for instance dichlorobenzene regioisomers. Reproducibility in retention time (RSD $< 2\%$) evidences the chemical stability of this new phase. Comparative experiments on PB-modified silica proved the key role of MWCNTs. The application to the analysis of real samples will be reported.

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DEVELOPMENT OF A MATHEMATICAL MODEL FOR ONLINE MICROEXTRACTION BY PACKED SORBENT UNDER EQUILIBRIUM CONDITIONS AND ITS APPLICATION FOR POLYCYCLIC AROMATIC HYDROCARBONS DETERMINATION IN WATER BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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In this work, partition equilibriums and extraction rates of different polycyclic aromatic hydrocarbons (PAHs) have been calculated by multivariate nonlinear regression from data obtained after microextraction by packed sorbent (MEPS) of 16 PAHs from water samples. PAHs can be considered of significant environmental concern due to their carcinogenic, mutagenic, and teratogenic effects (1). MEPS are very easy to use, fully automatable, of low cost, solvent-saver and fast in comparison with previously used method (2). The MEPS gas chromatography-mass spectrometry method (MEPS-GC-MS) has been optimized investigating the partitioning parameters for *a priori* prediction of solute sorption equilibrium, recoveries, pre-concentration effects in aqueous and solvent systems. Finally, real samples from sea, agricultural irrigation wells, streams and tap water were analysed. Detection ($S/N \geq 3$) and quantification ($S/N \geq 10$) limits were strictly dependent from the volume of water and methanol used during the extraction process. Under the experimental conditions used, these values range from 0.5 to 2 ng L^{-1} and from 1.6 to 6.2 ng L^{-1}, respectively. The reasonably good correlation between the logarithm of the partition MEPS-water constants (log $K_{meps/water}$) and the logarithm of the octanol-water partition coefficients (log K_{ow}) ($R^2=0.807$) allow a rough estimation of K_{ow} from the measure of $K_{meps/water}$. Furthermore, for each PAH, it is possible to evaluate the effect of different parameters, such as the volume of water or methanol, in order to improve the overall sensitivity of the method or the recovery ratio. Analyses with real samples prove that the method does not suffer from matrix effect, except for the tap water, where it has been hypothesized that the sodium hypochlorite, reacting with PAHs to produce chlorinated hydrocarbons that are not detected by the GC-MS in SIM mode set up for PAHs: further studies are currently in progress to verify the truthfulness of the proposed assumption.

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PHENOLIC ACIDS AND ANTIOXIDANT CAPACITY IN DURUM WHEAT AND ITS PRODUCTS

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The health-promoting effects of whole grains have been mainly attributed to the high contents of fiber and others bioactive compounds, including phenolic acids (PA). These compounds are mainly concentrated in the outermost layers of the grains and can be lost during the milling process, which determines a reduction of phytochemicals in refined grain products. Few studies have investigated the influence of milling and pasta making processes on the antioxidant properties of wheat grains and wheat-based foods. In the current study the effects of such processes on total antioxidant capacity (TAC) and PA content in durum wheat have been evaluated.

An Italian durum wheat cultivar (Duilio) was grown in an experimental trial in Montelibretti (Rome) during crop year 2010-2011. The grains after harvesting were milled by a pilot plant to obtain the main milling fractions (semolina, flour, coarse bran, fine bran). Pasta was made by using both semolina (traditional pasta) and wholemeal (wholewheat pasta). On all the samples obtained by technological processes the TAC, expressed as millimol Trolox equivalent antioxidant capacity per kg (mmol TEAC/kg), was determined following the direct method described by Serpen *et al* (1) and using ABTS radical; on the same samples the three forms of PA (free, conjugated and bound) were determined by a RP-HPLC method after an extraction performed according to the procedure proposed by Li *et al* (2), properly adapted to the different raw materials.

The milling process caused a sensible decrease both in the TAC and PA content (conjugated and bound forms) in semolina in respect to wholemeal. Also the pasta-making process negatively influenced the antioxidant compounds, determining an important decrease in the TAC values and in the bound form of PA content when semolina was processed into pasta. For wholewheat pasta no effect on TAC and on the conjugated PA was observed in respect to wholemeal, but only a light reduction for the bound PA.

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INSIGHTS INTO RETENTION MECHANISMS OF PERFLUOROALKYL ACIDS ON PERFLUORINATED SORBENTS. FLUOROUS AFFINITY CHROMATOGRAPHY AS A TOOL FOR ENRICHMENT AND ANALYSIS OF PERFLUORINATED EMERGING CONTAMINANTS

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The adsorption behavior of four perfluoroalkyl acids, including the environmentally relevant perfluorooctanoic acid, has been investigated on a straight-chain perfluorohexyl adsorbing material. The aim of this work is to provide original contributions to the potential use of fluorinated separation media for the analysis and/or the enrichment of perfluoroalkyl compounds in environmental samples. Water-acetonitrile-formic acid (0.1% v/v) mixtures were employed as mobile phases. An unusual U-shaped retention profile for all perfluorinated acids has been observed by changing the amount of acetonitrile in mobile phase and this has been correlated to the excess adsorption of the organic solvent, from binary water-acetonitrile mixture, on the adsorbent surface.

In addition, the concept of perfluoromethylene selectivity, defined in terms of ability of a chromatographic system to discriminate between molecules that differ by a single perfluoromethylene group, and traditional van't Hoff analysis were employed to describe the thermodynamics of phase transfer of analytes under study. Contributions to the Gibbs free energy for the passage of a perfluoroalkyl carbon from the mobile to the stationary phase have been evaluated and their meaning is discussed.

AQUAPHOTOMICS: DETERMINATION OF SALTS IN WATER BY NIR SPECTROSCOPY AND CHEMOMETRICS

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Aquaphotomics is a new term introduced to describe the concept of approaching water as a multi-element sysmte that could be well described by its multi-dimensional spectra (1). Indeed, information about water absorbance bands and absorbance patterns could provide a distinctive knowledge of water structures and intrinsic interactions between water and other components of the aqueous system (2). In particular, due to the energies and the nature of the transitions involved, NIR spectroscopy appears to be the preferred technique for aquaphotomic studies: indeed, the NIR spectrum of the solvent has been found to contain significant information about its solutes. To extract the relevant information about the nature of the interaction and/or the concentrations of the solutes, multivariate modeling by chemometric techniques plays a key role.

In this framework, aim of the present study was to investigate the possibility of using the aquaphotomic approach for the quantification of different inorganic salts in water. To this purpose, aqueous solutions of 6 salts ($AlCl_3$, KCl, $MgCl_2$, NaCl, KNO_3, and $NaNO_3$) at different concentrations were prepared and analyzed. Successively, calibration models for the quantification of the studied solutes were built using Partial Least Squares regression, after variable selection by Genetic Algorithms (GA) coupled to backward interval-PLS (biPLS, 3). This variable selection procedure, together with improving the predictive ability of the models, allowed a clearer interpretation of the results, highlighting the spectral regions characteristic of the interactions with the different solutes.

As far as the quantitative results are concerned, all the models were highly accurate and precise both in calibration and when validated on external test set, the average prediction error being around 1%.

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MULTIVARIATE STATISTICAL OPTIMIZATION OF BIOHYDROGEN PRODUCTION FROM CRUDE GLYCEROL

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Renewable resources are gaining importance as alternative pollution-free fuels for the future. Among them biodiesel has been used in diesel engines and heating systems for over 25 years. However, in the last years biodiesel production costs have been increasing due to the accumulation of crude glycerol as a byproduct. The development of processes to convert low-priced crude glycerol into higher value products is thus expected to add value to the production of biodiesel.

Conversion of glycerol can be obtained by either physico-chemical or biological methods. However, crude glycerol from biodiesel production is usually contaminated with water, methanol, soap and oil, leading to high purification costs when using traditional methods.

In this project the use of anaerobic fermentation to directly convert abundant and low-priced glycerol streams into higher value products is proposed.

In the first part of the project an enriched activity sludge that can effectively convert crude glycerol into bio-hydrogen was selected by an eco-biotechnological approach, in very strict conditions (minimal medium), using biodiesel-derived glycerol as the only carbon source. Principal Component Analysis allowed to deeply investigate the metabolic pathway: it shifted from a 1,3 propanediol-dominated to an ethanol type, with a concomitant increase of the hydrogen yield.

In the following part of the project, Design of Experiments (DoE) was applied to improve the ability of enriched activity sludge to efficiently convert crude glycerol into hydrogen. Plackett-Burman screening design identified initial glycerol concentration, temperature and initial pH as important variables. Box-Behnken design was then used for optimization. The maximum hydrogen yield of 0.96 molH_2 /molglycerol was estimated at temperature 37°C, initial pH 7.9 and initial glycerol concentration 15.0 g/L.

The last part of the project, that involves the scale-up and the study of the process in a continuous mode, is currently under investigation.

CRITICAL EVALUATION OF STRATEGIES FOR INTEGRATION OF DATA FROM DIFFERENT ANALYTICAL INSTRUMENTS: FUSION OF INFORMATION

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It is known that, in many applications, a single analytical instrument does not provide enough or it provides incomplete information about a property or characteristic of the sample that needs to be evaluated. In these cases, to combine information from different sources can be very useful.

The problem of the "fusion" of information, that is the combined use of data obtained from different analytical instruments, is very complex.

When considering fusion of different data, the first decision to be made is the level of fusion. On the lowest level, data fusion comes down to concatenating the matrices of measurements hence, all variables measured on the samples are simply put next to each other. There are indications in the literature that this is not the optimal way of fusion (1) because it is expected that some of the variables are not of primary importance for the problem or they could supply redundant information. Therefore, a variable screening method is used based on ideas of preliminary variable selection. The reduced data matrices are subsequently concatenated for obtaining the model. In this approach called mid-level fusion every data source is treated separately for pre-processing, scaling, and variable selection.

It is also possible to make separate models; the predicted response values can then be combined, e.g., by averaging. This is high-level data fusion. However, such combining of results has two disadvantages: (i) it does not give transparent models, i.e., interpretation of model results is difficult, and (ii) correlations between measurements in both blocks are not taken into account (2).

Other approaches have been suggested in this contest; for example the Principal Component Analysis (PCA) can be performed separately for each data set and the first Principal Components of each block can be considered and joined.

In this study, we consider also another strategy similar to the previous one, based on the possibility of combining the first LDA Canonical Variables or the first PLS Latent Variables instead of the first PCs, in classification and regression problems respectively.

A comparative study of these different strategies have been performed using real data sets.

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CHEMOMETRICS AND METABOLOMICS BASED ON LC-MS: FROM RAW DATA TO STATISTICAL MODELS

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The metabolomic approach can be defined as the analysis and interpretation of the global metabolic data expressing the response of living systems to genetic modification, pathophysiological stimuli and environmental influences. Untargeted LC-MS is often applied to obtain a holistic representation of the system under investigation. Large and complex data sets are produced and suitable tools are needed to extract the hide information. Chemometrics was successfully applied to untarget metabolomics studies both to process the collected data and to transform data into knowledge by statistical data modeling. Here we present a detailed discussion of the principal stages of the process leading from raw data to statistical models with particular focus on chemometrics. The aim is to provide a workflow where chemometric tools can improve the efficiency of the data processing and help the experimenter in the model building. Following Nicholson et al. (1) we introduce an experimental design where quality controls and blanks are included. In the data processing stage we discuss the use of component detection algorithms (2) to reduce the complexity of the spectra and remove the noise, the alignment step to match the corresponding peaks across multiple sample runs and the role played by the normalization (3) in the reduction of the systematic error by adjusting the intensities within each sample run. Also, we introduce the O2PLS (4) as regression technique to perform discriminant analysis and present useful visualization tools to interpret the statistical models. The proposed approach will be applied to obtain a detailed picture of the modulation of Corvina berry metabolites during ripening and post-harvest withering, based on an improved large-scale, untargeted analysis of the HPLC-ESI-MS chromatograms and a hypothesis-free analysis of subsets of the metabolite data matrix (5).

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COUPLING 2D-WAVELET DECOMPOSITION AND MULTIVARIATE IMAGE ANALYSIS

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Image analysis offers an invaluable help in the detection of surface defects in industrial productions, where the time factor in detecting anomalies in a large scale production is crucial. Also, multispectral images, where a spectroscopic signal is recorded at each pixel, can help in knowledge gaining tasks by giving the chemical information corresponding to a texture property. Wavelet transform (WT) is mainly used in image analysis as a preliminary step for denoising or compression or to extract textural features as a mean to obtain global image descriptors to be used for classification or properties prediction. In the present work, we develop an approach that uses the 2D-DWT (discrete wavelet transform) multiresolution advantage in the context of defects detection in single images. The basic idea is to combine the potentiality of the MIA approach with the wavelet decomposition scheme to take into account pixel correlation patterns. To this purpose, given a wavelet filter, the resulting blocks (Approximation, Horizontal, Vertical and Diagonal coefficients) from a 2D-WT decomposition of the image (DWT2 and SWT2 decomposition schemes are compared), applied separately to each channel, are used as different "versions" of the original image capturing the different patterns present in the image. By including approximations of every decomposition level, as many images as 4 times L (decomposition level) times N (channels) are obtained. These are unfolded to obtain a data matrix of dimensions: pixels × ($4 \times L \times N$). At this point the usual MIA approach is followed, afterwards constructing multivariate control charts for Hotelling-T^2 and residual sum of squares on the basis of one or few normal operating condition (NOC) images so that defects can be detected in faulty ones. The new proposal has been tested on different data sets, such as tile images with quite difficult to detect defects, oranges images corresponding to several damages and multispectral bread images to detect surface defect. The main goal, apart from a critical discussion of the most relevant aspects of this method, is to highlight the tipology of defects that can be handled by this method and how it may be used alternatively or complentary to the Bharaty and McGregor one, taking advantage of the unique features of WT, i.e. the fact that the different frequency content (related to texture) are depicted in disjoint subspaces.

CHARACTERIZATION OF OXOVANADIUM(IV) COMPLEXES WITH HYDROXYLATED CARBOXYLIC LIGANDS IN AQUEOUS SOLUTION

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This investigation deals with coordination compounds of oxovanadium(IV) with L-malic and L-tartaric acids in aqueous solution. The different binary systems are studied by potentiometric and spectroscopic techniques. Electronic paramagnetic resonance spectroscopy (EPR) at room temperature and molecular absorption spectrophotometry are employed. A speciation model is proposed for all the metal/ligand systems from potentiometric data and it is checked by means of a computational modelling (DFT = Density Functional Theory). For both the ligands, the formation of dimeric complexes, with the participation of deprotonated alcoholic groups in the coordination, is supposed. The best fit of potentiometric data is consistent with the formation of predominant dimeric species ($M_2L_2H_{-n}$) and scarcely relevant monomeric MLH_{-1} for malic and ML_2H_{-2} for tartaric acid. These results are well confirmed by EPR measurements which sustain the formation of dimeric complexes with small percentages of monomer. For each system investigated the individual spectrum of the relevant species is estimated and they are compared with the results of DFT calculations. The elaboration of potentiometric data leads to more than one speciation model, but utilizing spectroscopic data it is possible to select the model more feasible. DFT checked which kind of vanadyl complexes can match the speciation proposed, by examining equilibrium structures that are minimum in energy and that are able to reproduce the electronic spectrum. The elaboration of experimental data, together with a theoretical elaboration and the analysis of literature data (1-8), provided hypotheses on complexes structures in solution more reliable.

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INCLUSION OF ORGANIC ANIONS AND SELF-ASSEMBLY OF CALIXARENE CAPSULES IN WATER AT NEUTRAL PH

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The recognition, transport or transformation of anions is involved in almost every chemical and biochemical process (1). In the last decades, several ligands have been designed for anion recognition and calixarene-based macrocycles are among the most widely used synthetic receptors (2). Recently, we have shown that a tetracationic calixarene host is able to bind organic anions having different functionalities, shapes, sizes and charge at different pH values. The role of enthalpic and entropic contributions to the overall binding energy has been emphasized and it has been shown that such guests are bound to the receptor thanks to concerted hydrophobic and electrostatic interactions (3).

The anions that are best included have been employed for the design of templating agents for capsule formation. These structures may provide confined spaces to examine and manipulate the properties of encapsulated small molecules and reactive intermediates. We have shown that suitable dianionic gemini guests may trigger the formation of homodimeric capsules through combined hydrophobic and electrostatic interactions in water (4). A combination of different analytical techniques, such as ITC, ESI-MS, ^{1}H and DOSY NMR, allowed for the deconvolution of the host-guest equilibria and the determination of the species actually existing in solution. Such techniques, that probe different observables, cross-validate one another and demonstrated that significant amount of the capsules form in neutral aqueous solution. Our results suggest new strategies towards anion-templated molecular containers which may be employed as nano-shuttles for drug-delivery in highly competitive media like water.

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BINDING ABILITY OF GLUTATHIONE TOWARDS METAL AND ORGANOMETAL CATIONS

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Glutathione (*GSH*) is a tripeptide formed by the amino acids cysteine, glycine and glutamic acid. Despite its simple structure, it plays an important role in many biochemical processes that involve both animal and vegetable organisms. In particular, the presence in the molecule of several potential co-ordination sites, make it particularly useful in detoxification processes.

In order to evaluate the sequestering ability of glutathione and, therefore, its possible use as detoxificant agent in biomedical applications or to remove toxic metal ions from natural systems, we undertook a study on the interactions between this ligand and metal (Hg^{2+}, Pb^{2+} e Zn^{2+}) and organometal cations [CH_3Hg^+, $(CH_3)_2Sn^{2+}$, $(CH_3)_3Sn^+$, $(C_2H_5)_3Sn^+$, $(C_3H_7)_3Sn^+$]. For all systems, studies were performed by potentiometry at different ionic strength ($0.1 \leq I \leq 1$ mol L^{-1}) and temperature ($15 \leq t \leq 45$ ° C). In addition, UV-Vis and ^{1}H-NMR spectroscopy, in order to confirm the speciation models, and titration calorimetry, in order to give a complete picture of the thermodynamic properties, were used.

Speciation models of *GSH* -Hg^{2+}, -Pb^{2+} and -Zn^{2+} systems were quite similar with the formation of different ML_iH_j ($i = 1,2$; $j = 0,1,2$) species, together with ML(OH) and ML_2(OH), for Zn^{2+}. The stability of species was very different: as an example, for ML $\log\beta = 32.31$, 9.60 and 8.80, for Hg^{2+}, Pb^{2+} and Zn^{2+}, respectively (at $I = 0.1$ mol L^{-1} and $t = 25$ °C). Also for *GSH*-organometal systems, very similar speciation models were obtained, with formation of ML, MLH and MLH_2 species, together with ML(OH), for $(CH_3)_2Sn^{2+}$ only. Also in this case, the complexes with mercury(II) are more stable respect to those of other organometal cations.

On the basis of formation constants and speciation profiles, the sequestering ability of *GSH* towards different metals and organometals was quantitatively evaluated by determining an empirical parameter ($pL_{0.5}$) that numerically represents the ligand concentration [-log (total ligand concentration)] necessary to sequester 0.5 of metal ion fraction. The sequestering ability of glutathione was very high towards Hg^{2+} and CH_3Hg^+ and in particular experimental conditions significant also towards Zn^{2+} and Pb^{2+}.

POTENTIOMETRIC AND LASER DESORPTION MASS SPECTROMETRIC INVESTIGATION ON *trans*-HYDROXY-L-PROLINE AND Fe(III) EQUILIBRIA

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Hydroxyproline is an amino acid particularly abundant in the collagen, and its concentration in plasma and urines is considered an index of the metabolism of the collagen itself. It contains different donor atoms and for this reason it is considered a molecule with strong binding capacities (1, 2).
In this work the complex formation between the Fe^{3+} ion and L-*trans*-hydroxyproline, H_2L, has been studied at 298.15 Kelvin in 0.1 mol dm^{-3} in two different media, namely $NaClO_4$ and NaCl, in order to compare the results and to ensure that the ionic environment does not affect results.
The composition of the solutions was determined by potentiometric titrations by measuring with a glass electrode the competition of the L-*trans*-hydroxyproline for the metal and H^+ ions. The concentrations of ligand (C_L) and Fe^{3+} (C_M) were varied between ($1 \cdot 10^{-3}$ and $10 \cdot 10^{-3}$) mol dm^{-3}, and the ligand-to-metal ratio was varied between 1 and 10 ($1 \leq C_L/C_M \leq 10$). The hydrogen ion concentration was varied from $1 \cdot 10^{-3}$ mol dm^{-3} to incipient precipitation of basic salts which takes place in the range $[H^+] = 1 \cdot 10^{-4.5} - 1 \cdot 10^{-5}$ mol dm^{-3} depending on the specific ligand to metal ratio.
The general equilibrium can be written, schematically, for all systems as equation 1:

$$p\mathrm{Fe}^{3+} + r\mathrm{H_2L} \rightleftarrows \mathrm{Fe}_p\mathrm{H}_{-q}(\mathrm{H_2L})_r^{(3p-q)} + q\mathrm{H}^+, \qquad \beta_{pqr} \qquad (1)$$

Equilibrium formation constants, β_{pqr}, for the investigated ionic media are given. The speciation model and equilibrium data were determined on the basis of potentiometric evidences as well as the bonding sites by means of laser desorption mass spectrometry.

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ADSORPTION OF PHOSPHOROTIOATES PESTICIDES ONTO AMORPHOUS IRON (III) PHOSPHATE

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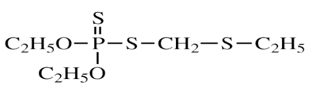
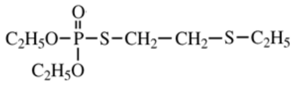
The widespread use of organochlorine pesticides in agriculture poses the problem of their distribution in the environment. Adsorption is one of the main mechanisms of retention of pollutants. Widely used pesticides are phosphorothioates aliphatic as phorate (A) and demeton−S (B).



$C_2H_5O-\underset{\underset{C_2H_5O}{|}}{\overset{\overset{S}{\|}}{P}}-S-CH_2-S-C_2H_5$

(A)



$C_2H_5O-\underset{\underset{C_2H_5O}{|}}{\overset{\overset{O}{\|}}{P}}-S-CH_2-CH_2-S-C_2H_5$

(B)

In this work has been investigated the adsorption of A and B onto amorphous iron (III) phosphate which is a component of soils and marine sediments [1]. The desorption has been investigated at 25 ° C in 0.1 and 1 mol/dm^3 NaCl as ionic media. The Γ function (which represents the moles of pesticide adsorbed per gram of $FePO_{4(s)}$) was determined by oxidation with bromine of the not adsorbed pesticide. The measurements carried out on B show that it is not adsorbed onto the solid phase under the experimental conditions investigated. The adsorption isotherms of A follow the Langmuir model. In particular, Γ is independent on pH as shown in Figure 1. Analyzing the data according to the model of surface complexation, A interacts with the solid phase ($>S(OH)_2$) according to the equilibria: $>S(OH)_2 + A = >S(OH)_2A$ logK = 3.4 ± 0.1. valid at 25 ° C in 0.1 mol/dm^3 NaCl. The adsorption of phorate is not dependent on the ionic strength of the solution as shown in Figure 2. This is in agreement with the formation of a complex surface to the internal sphere [2].

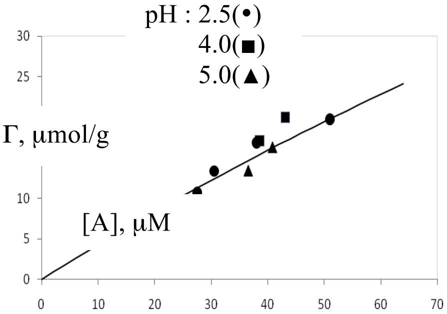


Figure 1

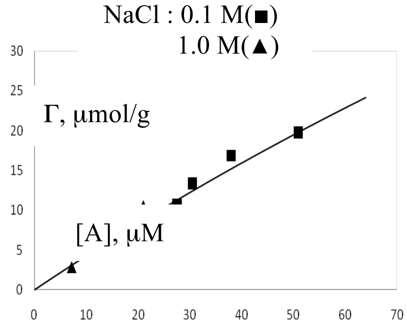


Figure 2

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INORGANIC COMPONENTS IN HONEYS AS POTENTIAL INDICATORS OF BOTANICAL ORIGIN AND OF ANTHROPOGENIC ENVIRONMENTAL POLLUTION

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Aim of this study was to investigate the quali/quantitative profile by IC (Ion Chromatography) technique of the inorganic constituents (cations: Na^+, Ca^{++}, Mg^{++}, NH_4^+ and anions: Cl^-, Br^-, SO_4^{--}, NO_3^-, PO_4^{3-}) and compare the results obtained from honeys of different Italian Regions (Lombardy, Piedmont, Sardinia, Calabria, Tuscany) with those from countries of the Western Balkan area (Slovenia, Croatia, Serbia, Kosovo, Macedonia and Albania). The mineral concentrations of the two honeys groups were further analyzed by multivariate statistical techniques such as principal component analysis (PCA) and hierarchical cluster analysis (HCA). The results provide a detailed and exhaustive view of the ionic composition of the different honeys, up to now never reported. They can be applied for the detection of differences in mineral concentrations among honeys allowing, from a botanical poin of view, a sharp differentiation between nectar honeys and arboreal/honeydew honeys (discrimination of the floral source) and used as an index of the purity of the matrix or of its fraudulent adulteration with sugars, syrups, etc.. Finally the obtained results can be used to individuate the natural (by bees) or artificial blending between different honeys. Furthermore the multivariate analysis allows to demonstrate the potential of honey as bioindicator of the distribution of impact of various environmental pollutants of industrial and urban origin (Br^-, SO_4^{2-} and PO_4^{3-} contents) which show a steep increase in honeys of Western Balkan area. In particular for what concerns arboreal honeys the concentrations of SO_4^{2-} and of PO_4^{3-} in honeys from Balkan area were three times greater than those present in the Italian ones (SO_4^{2-}=90.3±60.3 ppm vs. SO_4^{2-}=29.6±20.8 ppm; PO_4^{3-} =772.6±530.3 ppm vs. PO_4^{3-}=222.0±74.2 ppm respectively). Br^- was undetectable in almost all the Italian honeys and its presence in Balkan honeys could be attributed the use of methyl-bromide for agricultural use. The findings reported in this presentation fit into a research program that aims to typify the quality of honeys in different countries.

PRIMARY METABOLISM OF BERBERINE IN HUMAN: CORRELATION BETWEEN PHYSICOCHEMICAL PROPERTIES AND PLASMA LEVELS BY HPLC-ES-MS/MS

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Berberine (BBR) is a natural isoquinoline alkaloid belonging to therapeutic agents called "nutraceutical" which don't require conventional drug approval regarding pharmacokinetics, metabolism and safety. Considering the uncontrolled large use of this substance in recent times, it's very important to have a complete view of its fate after oral administration. We developed and validated a HPLC-ESI-MS/MS method for the identification and quantification of BBR and its main primary metabolites (Berberrubine M1, Thalifendine M2, Demethyleneberberine M3, Jatrorrhizine M4) in plasma. These metabolites have been synthesized for properly quantification. This method was applied to a pharmacokinetic and activity studies after chronic administration and we found that level of BBR in plasma was very low, BBR(~1.4 ng/mL), M1(~2.3 ng/mL) and M2 (~1.2 ng/mL) and much less for M3 (≤0.5 ng/mL) and M4 (1.9 ng/mL). After chronic feeding for 3 month at a daily dose of 15mg/kg die BBR is able to reduce the plasma cholesterol in patients with hyperlipidemia. Moreover BBR metabolites are also potentially pharmacologically active since accumulate even more than BBR itself. To better understand the overall pharmacokinetics and the structure-activity relationship the main physicochemical properties in aqueous solution have been measured including the lipophilicity ($LogP_{o/w}$), *pKa*, and the binding affinity with albumin of BBR. The lipophilicity evaluated as 1-octanol/water partition coefficient ($LogP_{o/w}$ pH=4.5) is very different among the BBR and its metabolites (BBR -0.45, M1 0.85, M3 0.33, M4 0.41) in spite a quite similar chemical structures. The positive correlation between plasma levels and lipophilicity suggest that M1, with the higher *LogPo/w* is present in higher concentration as a results of an efficient intestinal absorption by passive diffusion. The lower plasma levels of the other metabolites is the result of a lower $LogP_{o/w}$. The uncommon metabolism of BBR which produces metabolite even more liphophilic such as M1, M3, M4 suggest that these molecule are potentially active like or even more that BBR.

COMPREHENSIVE PROFILING OF CAROTENOIDS AND FAT-SOLUBLE VITAMINS IN MILK FROM DIFFERENT ANIMAL SPECIES BY LC – DAD – MS/MS HYPHENATION.

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Simultaneous analysis of fat-soluble micronutrients is a challenging task, due to the different sensitivity of these substances towards light, oxygen, heat and pH. For the same reason, the literature has reported few methods for the multi-vitamin analysis, especially if concerning complex matrices such as foods (1,2). This paper describes a novel and efficient analytical method to define the fat-soluble vitamin and carotenoid profile of milk from different animal species. Until now, little has been known about both the carotenoid composition of bovine milk (3,4) and the fat-soluble micronutrient fraction of buffalo, sheep, goat and donkey's milk.

In this work, overnight cold saponification was optimised as simultaneous extraction procedure. Analytes were separated by non-aqueous reversed-phase (NARP) chromatography: carotenoids (all-*trans*-lutein, all-*trans*-zeaxanthin, all-*trans*-β-cryptoxanthin, all-*trans*-β-carotene) on a C_{30} column, while fat-soluble vitamins (all-*trans*-retinol, α-, γ-, δ-tocopherols, ergocalciferol, cholecalciferol, phylloquinone and menaquinone-4) on a tandem C18-column system. The feasibility of the whole strategy was then verified analysing the different kinds of milk. Besides the above-mentioned target analytes, the DAD-MS combined detection allowed the provisional identification of other carotenoids on the basis of the expected retention times, the absorbance spectra and mass spectrometric data, without support of authentic standards.

Retinol and α-tocopherol were the most abundant fat-soluble micronutrients, especially in small ruminant milk, and were the only ones found in donkey milk along with γ-tochopherol. All the milks from ruminants also proved to be a good source of vitamin K vitamers (phylloquinone and menaquinone-4). Bovine milk distinguished itself because it showed a high amount of β-carotene and a large variety of carotenoids which, with the only exception of all-*trans*-lutein, were completely absent in milk samples from the other species.

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PRELIMINARY STUDY ON QUANTIFICATION OF α_{S1}-CASEIN VARIANTS IN GIRGENTANA GOAT BREED BY DIRECT CHROMATOGRAPHIC ANALYSIS OF MILK

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Goat α_{s1}-casein is a highly polymorphic protein, coded by *CSN1S1* gene. Nowadays, several alleles were identified and associated with different levels of α_{s1}-casein in goat milk. Polymorphisms at α_{s1}-casein locus have been shown to affect not only the quantity of this casein in goat milk, but also the structural and nutritional characteristics (hypoallergenic properties) and technological properties of the milk (1). The aim of this work was to separate and quantify the most common allelic variants of α_{s1}-casein in milk of Girgentana goat breed, a Sicilian autochthonous breed, and to evaluate the effect of α_{s1}-casein polymorphisms on casein content.
The *CSN1S1* A/01, B/E, F, and N alleles were simultaneously investigated by PCR-RFLP (2). AS-PCR was used for the detection of the *CSN1S1* E (3) and 01 alleles (4). Milk samples were prepared following the method proposed by Bobe et al. (5) and analyzed by RP-HPLC method (6). A reversed-phase analytical column C8 (Zorbax 300SB-C8 RP, 3.5µm,300Å, 150×4.6 I.D.) was used and the detection was made at a wavelength of 214 nm. The procedure was developed using individual raw milk samples of Girgentana goats. For calibration experiments, pure genetic variants were extracted from individual milk samples of animals with known genotypes, considering that commercial standards for goat allelic variants were not available. In particular, were used animals with AA, BB, FF and NN homozygous genotypes. Method validation consisted in testing linearity, repeatability, reproducibility and accuracy. A linear relationship between the concentrations of proteins and peak areas was observed over the concentration range, with low detection limits. Repeatability and reproducibility were satisfactory for both retention times and peak areas.

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APPLICATION OF DIFFERENT TECHNIQUES TO DETECT IRRADIATED FOOD AT THE PRODUCT MARKETING STAGE

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Food irradiation can be used to increase the microbiological safety and to extend the shelf life of foods. Community legislation (1999/2/CE e 1999/3/CE) states that any authorised food or food ingredients must be labelled with the words “treated with ionizing radiations” or “irradiated” and that every year each Member State, has to carry out checks at marketing stage. This work reports on the results of analytical controls of 452 foodstuff samples over the period 2006-2011 and analysed with 4 different screening and confirmatory techniques: Photostimulated Luminescence (PSL), DNA Comet Assay, Thermoluminescence (TL) and Electron Spin Resonance (ESR). All of analytical checks were carried out implementing six European Standards: EN 13751 (1) for shellfish, herbs, spices and seasonings, EN 13784 (2) for food containing DNA, EN 1788 (3) for food from which silicate minerals con be isolated, EN 1786 (4) for food containing bone, EN 1787 (5) for food containing cellulose, EN 13708 (6) for food containing crystalline sugar. Foodstuffs were collected, analyzed with suitable methods and distinguished in six food categories: meat products, fish products, herbs spices and seasonings, fruits, vegetables and others. Results showed that 19 samples, imported from Vietnam and China, including frog legs, clams, shrimps, cuttlefish, tofu, squids, white pepper and coriander, were found non compliant with European label requirements. As a consequence Italian Ministry of Health sent several RASFFF (the Rapid Alert System for Food and Feed) notifications. In conclusion to further safeguard the consumer health and right of choice it is important to develop and optimize analytical protocols along with the adoption of selected sampling plan.

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THE EVOLUTION OF ENVIRONMENTAL ETHICS FOR A SUSTAINABLE WORLD

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Evolution of Environmental Ethics starting from ’60 years of the last century is traced: premise is the vital role of chemicals for welfare of modern societies, but, in the same time, effects on environment were observed. This determined in the world development of national, regional and international regulation of chemicals issued in EU to REACH regulation (EC 1907/2006). In the same time perception of a variety of technological risks was developed [1]. The answer by International community was the setup of Responsibility [2] and Precautionary principle, (Rio World Summit, 1992) and Sustainable development [3-5]. Science and Technique should start from Nature and suitable green chemistry concepts were thus developed (eg “ecological footprint” and “virtual water”). An integrated approach between ethics, science, education and politics is moreover essential with a new roles of green analytical sciences, e.g of chemometrics [4-6]. The approach called “scientific proceduralism” can thus offer a necessary integration between scientists and lay people [6]. Ethical rules to be followed in the case of choices between exhaustive technical alternatives must singled out to avoid the so called “naturalistic fallacy” [7]. We expect new directives toward sustainability development from the forthcoming Rio conference (Rio+20 Earth Summit) [8].

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APPLICATIONS OF ANALYTICAL PYROLYSIS TO THE DEVELOPMENT OF FUELS AND CHEMICALS FROM BIOMASS

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In setting up analytical procedures, sample preparation is a common step which may require the use of toxic or corrosive substances with potential environmental and health hazard. Analytical pyrolysis (Py) coupled with GC-MS is a solventless technique requiring minimal sample preparation and reagent use. Methods based on Py/GC-MS have been developed with a higher degree of "greenness" in comparison to wet analysis (1).

Py/GC-MS has been largely applied to the characterisation of complex and heterogeneous macromolecules deriving from the thermal and environmental degradation of biopolymers. Therefore, it represents a valid approach to study the liquid deriving from the thermochemical conversion of biomass (bio-oil) finalised to the production of biofuels and new chemicals (2).

However, conventional Py/GC-MS is flawed by several factors (e.g. mass transfer, aerosol formation, memory effects) limiting its ability to provide an overall picture of the molecular composition of bio-oil without the aid of laborious solvent fractionation procedures. To the end of improving Py/GC-MS, we have developed a new approach based on SPME sampling of pyrolysis products evolved from the sample heated at sequentially increasing temperatures (stepwise). Stepwise Py/SPME/GC-MS has been applied to the characterisation of complex bio-oils obtained from the thermochemical treatment of microalgae (3). We have now demonstrated that the method can be combined with on-fiber derivatisation expanding its potential to the analysis of polar constituents, such as thermal degradation products of polysaccharide and proteins.

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MICROREACTOR TECHNOLOGY: A GREEN ANALYTICAL TOOL FOR THE STUDY AND CHARACTERIZATION OF COMPLEX REACTIONS

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Green analytical chemistry processes aim at minimizing the effects of pollution agents in the human health and nature through the design of new processes or new tools. One of the main trends to achieve this objective is the introduction of new technology based on miniaturized methods that allow for a reducing of reagents and/or materials, on the one hand, and that also permit the gathering of information necessary in the transition from laboratory- to production-scale, on the other.

In this presentation, we will focus on microreactor technology (in particular, packed-bed microreactors) as a tool for investigating complex catalytic reactions. Modern approaches for the characterization of the kinetics and the thermodynamics of the catalytic processes in flow-mode will be discussed through the study of slow and fast model reactions.

Technical and instrumental expedients as means for process optimization and automation will be presented with particular emphasis to the preparation of new catalytic supports through green organic chemistry strategies.

ON THE MEASUREMENT OF THE PHOTOCATALYTIC ACTIVITY FOR THE ABATEMENT OF GASEOUS SPECIES

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The possible market for photocatalytic materials, in the form of powders, built-in powders, thin films and nanostructured materials, as well as devices directed to specific applications, is estimated in exponential growth (1).
The central problem for standardization of photocatalytic efficiency of whatever material is the rate evaluation. Among the used, proposed or approved protocols of standardization of gas/solid activity there are different reactor configurations (plug (PFR, Plug Flow Reactor) or continuous flow (CSTR, Continuous Stirred-Tank Reactor) as well as batch reactors or circulating fluidized bed) using different kind of substrates (VOC , NO, mixture of $NO+NO_2$, NO2, acetaldehyde, toluene, formaldehyde, methyl mercaptan). However, depending on reactor type and conditions, the analytical measurements gives different calculated rates.
As "Analytical chemistry is the science of obtaining, processing, and communicating information about the composition and structure of matter" according to the definition of ACS (2), and because "...measurements [of analytical chemists] are used to assure compliance with environmental and other regulations", in this broad meaning the analytical chemist has to correctly measure the concentration as a function of time or the inlet and outlet concentrations in the above reactors, but also to find the right way to measure what regulation needs. In this framework, the basic equations governing the above reactors and the rate expression for them are here presented. Experiments using either the abatement of NO and toluene as model pollutants 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. A CSTR configuration is superior to the standardized PFR used in the ISO test 22197-1:2007, as the resistance to mass transfer, which leads to under-evaluate the rate, can be reduced by inside forced ventilation. Consequently, it gives an assessment of the photocatalytic rate more close to the actual surface one. The rate for CSTR at steady state must be calculated as $r(C_o)= C_o F \eta /(1-\eta)$, where η is the conversion. The revision of the standard methods ISO 22197-1:2007 and UNI 11247-2010 for photocatalytic materials is needed.

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BINDING STUDY OF HEPARIN FROM DIFFERENT SOURCES TO ANTITHROMBIN BY AFFINITY CAPILLARY ELECTROPHORESIS

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Heparin, a highly sulfated polydispersed glycosaminoglycan (GAG), is the most widespread clinical anticoagulant; it binds antithrombin III (AT), a member of serine proteinases superfamily, accelerating its antagonist effect on blood coagulation. The binding interaction with AT is an important aspect of the characterization of physicochemical properties of GAGs. With the aim at profiling several clinical and experimental heparin batches from different sources (porcine, bovine and ovine mucosa), a quantitative AT-heparin binding investigation was undertaken by means of Affinity Capillary Electrophoresis (ACE).

In dynamic-equilibrium ACE, the electrophoretic mobility of the receptor (AT), analysed in a BGE containing the ligand (the considered GAG), is correlated to ligand concentration and binding constant. In particular, a 20 mM sodium phosphate, pH 7.4 buffer (the BGE) was chosen as the neat medium and the experiments were carried out in a highly hydrophilic poly(vinyl alcohol) coated capillary (effective length 8.5 cm). The applied sample consisted of the receptor AT (0.50 µM) and phenylacetic acid (PAA; 10.0 µM) used as a reference compound. The samples were run in triplicate at each of the studied concentration levels of the ligand (heparin, 1.0 – 10.0 x 10^{-7} M) supplemented to the BGE. The migration time ratio of PAA to AT was assumed as the chemical response to be correlated to the ligand concentration and the binding constant estimation was based on the application of a nonlinear regression method (rectangular hyperbola). The average analysis time was in the order of 4 min. Under these conditions, 18 heparin samples were analysed and their binding constants (Kd) were found between 13 and 54 nM (SD $\leq \pm$ 1.2; n = 3; coefficient of determination $r^2 \geq$ 0.98) with significant differences depending on the origin.

Correlation of the Kd values to in vitro anti-factor Xa and anti-factor IIa potencies was evaluated. Both heparin activities demonstrated to be closely related to Kd, independently from the kind and the source of the sample. Commercial samples from the same manufacturer showed a certain dispersion around the fitting, but the use of the mean point of the cloud significantly improved the coefficient of determination.

STABILITY CHARACTERISATION VIA ISOTHERMAL AND SCANNING CALORIMETRY

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The stability or instability of a single material or of a mixture is usually associated with a heat exchange with the surrounding environment. Calorimetry thus has been proposed as an optimal choice to characterize stability in many fields (1). In this work we want to show a few examples on what information can be obtained with an isothermal and a scanning calorimeter about the stability of a mixture or a pure material. The amount of heat released by sodium percarbonate is extremely important for the chemical industries employing this material (2). The right choice of an eccipient for a drug is also driven by the requirement that the two molecules do not show any mutual interaction (3). Finally, the interaction of an enzyme, like RNase, with another molecule, like 2' CMP, can increase the stability of the former molecule, and this can be assessed using both titration calorimetry and differencial scanning calorimetry (4).

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CID/ETD TANDEM MASS SPECTROMETRY FOLLOWING NANO LIQUID CHROMATOGRAPHY FOR CHARACTERIZING PHOSPHOPROTEINS

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Mass spectrometry is currently the method of choice to detect changes in protein phosphorylation and to identify the position of specific phosphorylation events. However, even with recent advances in mass spectrometry instrumentation, the detection and identification of phosphorylation sites is a challenge. In this study, the advantages of using electron transfer dissociation (ETD) combined with collision-induced dissociation (CID) as orthogonal fragmentation techniques for global phosphoproteomics analysis is demonstrated. ETD represents one of the most recent and significant advancements in tandem mass spectrometry for the identification and characterization of post-translational modifications (1,2); nevertheless analytical methodologies have not been established yet. Therefore, the complementary nature of CID and ETD fragmentation has been exploited for a comprehensive peptide characterization, and a new protein digestion protocol has been developed. The effect of different proteolytic procedures using chymotrypsin, trypsin, a combination of both, and Lys-C, has been carefully evaluated in terms of coverage percentage, the number of identified peptides and their size and charge state. A systematic comparison between CID and ETD is shown for the analysis of phosphopeptides deriving from digestion of casein standards. The best results have been achieved with a mixture of trypsin and chymotrypsin, combined with CID and ETD operating in an alternating mode. This approach has allowed the obtainment of a high number of essential information to identify peptide sequences and localize the phosphorylation sites. Not phosphorylated peptides have been sequenced and identified by CID and ETD fragmentation spectra, generated in parallel by the same precursor, consequently a decrease of false positive rate of protein identification was ensured. ETD experiments have allowed the acquisition of several spectra of high quality, and a confirmation of spectral information for the phosphorylation study has been reached. The potential of this novel strategy has been tested by the analysis of both αS- caseins from bovine milk, and non-phosphorylated peptides from bovine serum albumin, as the control system.

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TOWARDS TOTAL AND FREE IRON(III) SENSING

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Our intent was to set up a solid mainframe able to sorb iron(III) from a test solution where strong iron chelators are present. From the sorbed fraction, the total iron and the free iron concentrations are calculated (1). We selected DFO (deferoxamina) as candidate active centre MS (mesoporous silica), as solid phase and a novel *one pot* synthesis (reported in fig. 1) to obtain the sensor.

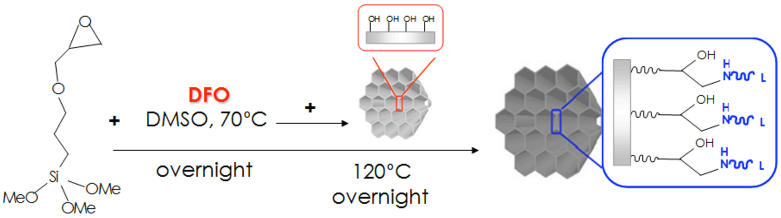

Figure A1- Synthesis *one pot* of DFO immobilized mesoporous silica.

The *one pot* synthesis was optimized, according to experimental design strategy. We applied a full factorial design 2^k, where k denotes the number of variables (temperature, type of silica -different pore sizes were considered- and type of DFO (mesylate salt or neutral) to find conditions that provide the maximum adsorption capacity q_{max}. The best yield was obtained under temperature at low values of 90°C, MCM-41 small pores silica and DFO in its neutral form.

In the second part of the project, we have explored the sorbing properties of the optimized product. A series of experiments in controlled KNO_3 and urine media demonstrates that in a wide pH range the complex FeHL is formed between the anchored DFO and the sorbed Fe(III) ions. The exchange coefficient, $\log\beta_{ex}$ = 40.1(2.5), is in pretty good agreement with the property of the ligand in solution.

Finally, urine samples, simulating patients with overloading disease under chelation therapy, were titrated with the solid phase to assess the total iron concentration and the complexation degree, according with a consolidate method (1). Even if further tests must be performed, the encouraging results demonstrate the consistency of the strategy proposed for speciation study.

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ADSORPTION OF SELECTED PHARMACEUTICALS BY ZEOLITES

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The limited removal of pharmaceuticals under conventional sewage treatments requires improvement of advanced treatment technologies to avoid environmental pollution [1, 2]. One of the possible tertiary treatment is adsorption process, using zeolites as sorbent materials.

Previous studies have demonstrated that zeolites are efficient adsorbent materials for water remediation [3]. In the present work, the influence on the adsorption of both zeolite characteristics (i.e. framework type, hydrophobicity index, thermal treatments) and physico-chemical parameters of the drug solutions (i.e. pH, ionic strength) are investigated. In particular the adsorption properties of two different zeolites (i.e. Y and Beta) with respect to drugs belonging to various therapeutic classes, having different molecular dimensions and physico-chemical features, have been considered. Adsorption isotherms and thermogravimetric analysis revealed that the amount of pharmaceuticals embedded inside the zeolite framework is related to lattice structure but it as also strongly influenced by zeolites hydrophobicity and by the thermal treatments. The adsorption capacity of a given materials depends also on the pH, and on the ionization constant of the drug.

Finally, X-ray diffraction patterns indicated that the crystal structure is markedly modified by the adsorption of pharmaceuticals, proving that the adsorption of the drugs occurs inside the zeolite channel system. It has been found that drug release from porous materials can be employed for controlled drug delivery systems (i.e. devices that enable for an accurate control the rate at which drug molecules are delivered into the bloodstream). In such application, the adsorbent material plays a crucial role in controlling the release of drugs in the body in order to maintain the concentration within the optimum range, hence to improve the therapeutic efficacy and to reduce toxicity. To evaluate the capability of zeolites in drug delivery application, drug release experiments were carried out. The results demonstrate that zeolites are also promising materials for controlled delivery systems.

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HIGH-RESOLUTION MULTY PROXY RECORD OF CLIMATIC AND ENVIRONMENTAL CONDITIONS DURING THE HOLOCENE IN THE EASTERN ITALIAN ALPS USING A NOVEL XRF AND ICP-MS CALIBRATION METHOD

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Ombrotrophic bogs are hydrologically isolated from the influence of local groundwaters and receive their nutrients exclusively from the atmosphere by dry and wet deposition. For that reason these systems have a great potential for recording the chronology of past atmospheric deposition. Unlike glacial archives, which reflect integrated long-range metals input, peat bogs can record dust supplied by local and regional sources, allowing the study of regional and local scale variability. Here we present the first data from Italian peat records reconstructed from a 13,221 yr cal BP peat core. The aim of our work is to reconstruct spatial and temporal variation of past climate and environmental conditions by a novel multi-proxy method characterized by high-resolution geochemical (trace elements, rare earth elements, Pb isotopes, organic carbon, humification) and pollen analysis using the Danta di Cadore 46°34'16'' N 12°29'58'' E and Coltrondo 46°39'28'' N 12°26'59'' E peat bogs from the north eastern Italian Alps. We propose a high resolution analytical approach based on the analysis of split sediments with the Avaathech XRF Core Scanner followed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis: we quantified major and trace elements in the peat profile, moreover we obtained lightness and colour parameters on the images recorded by a digital colour line scan camera connected to the XRF scanner. Conversion of element intensities measured by XRF core scanner to element concentrations is essential for quantitative applications involving mass-balance and flux calculations. Intensities of our XRF results were calibrated with control specimens taken from the same core at strata corresponding exactly to core-scanner measurements: total concentrations of elements were determined by using destructive tecnique employing microwave-assisted HF–HNO_3 digestion of peat. Samples treated in this way were subsequently analyzed by ICP-MS. Our determinations of specific element concentrations describe the trophic status of the peat profile and, together with a reliable age model combining ^{14}C dating with the independent ^{210}Pb dating, allow the reconstruction of rates and predominant sources of a variety of atmospheric trace elements. To our knowledge, this is the first attempt to provide quantitative geochemical interpretation of XRF core scanner data for this type of deposit. The results provide environmental information which is currently lacking in the north-eastern Italian Alps and more generally in northern Italy where a complete quantitative environmental record of major and trace elements from prehistory to the present has not yet been constructed.

ENHANCED HOLLOW FIBER FLOW FIELD-FLOW FRACTIONATION FOR THE ANALYSIS OF COMPLEX PROTEIN SAMPLES

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Flow field-flow fractionation (F4) is well-suited to the analysis of complex protein mixtures without modification of the protein conformation and protein-protein interactions. This is because (a) F4 is based on a gentle separation mechanism, (b) it can utilize almost any aqueous solution as carrier, and (c) no stationary phase is involved.

Hollow-fiber F4 (HF5) is the microvolume, tubular variant of F4 [1]. Compared to flat-channel F4, HF5 shows additional and unique advantages: (a) low channel volume that reduces sample dilution, (b) possible disposable usage that eliminates the risk of run-to-run sample carry-over, and (c) low flow rate conditions that are ideal for on-line coupling with MS for applications in proteomics [2].

In this work we present an improved version of HF5 technology [3] for the separation of complex protein samples. A mixture of four standard proteins, which range from 30 to 670 kDa in molar mass, is used as a first model sample. Performance of the HF5 method is found comparable to that of commercial F4 methods in terms of efficiency, resolution and selectivity. When applied to IgG samples at different aggregation state, HF5 is found able to fractionate and quantify the different oligomeric forms. The effect of sample load and mobile phase composition on fractionation is discussed.

HF5 online coupled with multiangle light scattering (MALS) is then applied to size fractionation and characterization of cell lysates from mice of different ages. The protein aggregates related to ageing processes are characterized and fractionated for further proteomic analysis.

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RAPID DESORPTION ELECTROSPRAY IONIZATION-HIGH RESOLUTION MASS SPECTROMETRY-BASED METHOD FOR THE ANALYSIS OF MELAMINE MIGRATION FROM MELAMINE TABLEWARE

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Migration of melamine into foods from melamine-made tableware has been object of recent Rapid Alert System for Food and Feed (RASFF) notifications. Legislation in the European Union was put in place to guarantee the safety of food contact materials in terms of specific migration limit (2.5 mg/kg for melamine (1)). In this context, a rapid and sensitive desorption electrospray ionization-high resolution mass spectrometry (DESI-HRMS) method was developed and validated for the determination of migration of melamine from plastic materials into food. The migration test was performed using acetic acid 3% (*w/v*) as food simulant.

Evaluation of the DESI parameters (i.e, support, scanning mode, geometrical configuration and operating conditions) and the use of an orbitrap mass analyzer allowed to achieve significant improvements in terms of selectivity, accuracy and sensitivity, obtaining detection and quantitation limits at low µg/kg level. A LC-ESI-MS method was developed for confirmatory purposes. Both methods were applied to melamine tableware available on the children's market in Italy in order to assess their compliance with the law. Different concentration levels were found in new and used tableware (mg/kg *vs* µg/kg level). Quantitative results obtained applying the DESI-MS method were in good agreement with those provided by LC-ESI-MS, thus proving reliability of DESI-MS as rapid screening technique for the study of melamine release from plastic materials.

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SURFACE CHEMISTRY OF Ni-FREE STAINLESS STEEL

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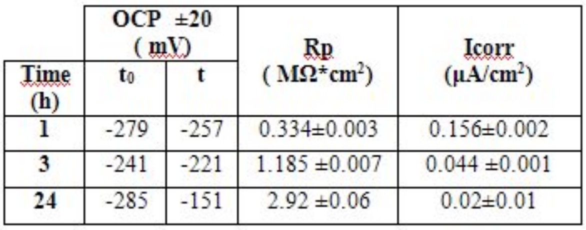
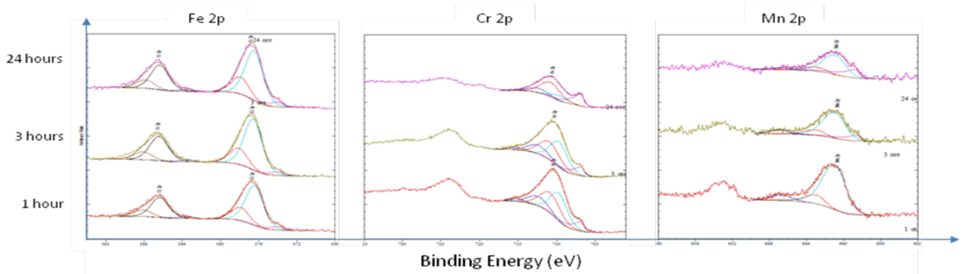
Nowadays there is a strong demand to replace CrNi stainless steels used for biomedical applications such as brackets in orthodoncy with a new generation of more biocompatible austenitic, nickel-free alloys. The aim of this work is the assessment of the substances that leach from the steel and the investigation of the growth and stability of the surface film formed on DIN 1.4456 Ni-free stainless steel (18% Cr, 18% Mn, 2% Mo) in artificial saliva (pH 7.9) at 37°C by electrochemistry and XPS surface analyses. So far the data available in the literature refer to tests carried out at ambient temperature. Previous works of this research group [1] have shown that Ni-free stainless steel immersed into 0.1M NaOH solution form a passive film that changes its composition with immersion time. These results are confirmed in the present investigation: the corrosion current values decrease from 1 to 24 hours exposure time to the solution (see Table) and the passive film becomes more protective and thick. XPS provides evidence that the surface film formed is mainly composed of oxides and hydroxides of Fe (III), Cr(III) and Mn (see figure). Fe (II) amount in the passive film decreases with immersion time in favor of Fe (III) oxide (Fe_2O_3) and hydroxide (FeOOH). Cr as well is first present as Cr_2O_3 and turns to $Cr(OH)_3$ for longer contact time to artificial saliva.



Time (h)	OCP ±20 (mV)		Rp (MΩ*cm²)	Icorr (µA/cm²)
	t_0	t		
1	-279	-257	0.334±0.003	0.156±0.002
3	-241	-221	1.185 ±0.007	0.044 ±0.001
24	-285	-151	2.92 ±0.06	0.02±0.01

The results will be discussed in comparison with those obtained on the same alloy at ambient temperature [2,3].



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PE-CVD AS A POWERFUL TOOL FOR P3HT SURFACE MODIFICATION IN EGOFET BIOSENSORS DEVELOPMENT

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Organic Field Effect Transistors (OFET) based sensors are widely being studied because of their suitability for cost-effective mass fabrication on flexible substrates (1). The sensitivity and selectivity of OFETs can be increased by integrating biological receptors specific for the analyte to be detected (2, 3). Although many methods for bio-molecules immobilization exist, the integration of bio-receptors on the active area of OFETs is a major challenge. In this study, a radio frequency (RF, 13.56 MHz) Plasma Enhanced Chemical Vapor Deposition (PE-CVD) process was employed to functionalize the poly(3-hexylthiophene) (P3HT) organic semiconductor surface of Electrolyte Gated Organic Field Effect Transistor (EGOFET) devices (4, 5) with hydrophilic organic coatings characterized by –COOH groups. Acrylic acid vapors were used to feed the discharges. Different plasma deposition times were evaluated to optimize the deposition period and grant optimum electrical performance of the EGOFETs without affecting the bulk properties of the material. The surface chemical composition of P3HT before and after PE-CVD was measured by X-ray photoelectron spectroscopy (XPS). XPS data revealed the presence of carboxyl functionalities on the plasma treated P3HT surfaces even weeks after plasma deposition. The effect of annealing on the electrical performance of the EGOFETs before and after PE-CVD was also investigated. Carboxyl groups present on the coatings can serve as anchor sites to immobilize bio-receptors onto the EGOFET devices and further functionalize them for biosensors development.

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ELECTROCHEMICAL IMMUNOASSAY FOR CA125 DETECTION BASED ON SILVER-ENHANCED GOLD NANOPARTICLE LABEL

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In this work, a sensitive electrochemical immunosensor for ovarian carbohydrate antigen 125 (CA125) has been developed. CA125 is a mucin-like glycoprotein, greater than 200 kDa, which was first detected over 30 years ago using the OC125 monoclonal antibody. It is mainly associated with diagnosis and prognosis of ovarian cancer and presents a clinical threshold of 35 U/mL. The immunosensor is based on the precipitation of silver on colloidal gold labels which, after silver metal dissolution in an acidic solution, was indirectly determined by anodic stripping voltammetry (ASV) at a modified screen printed electrode. In this method, O-aminobenzoic acid (O-ABA) was first electropolymerized onto a graphite screen-printed electrode (GSPE). The captured monoclonal antibody was then immobilized by carboxyl groups of the polymer using EDC/NHS coupling reaction. Subsequently, the immunosensors were incubated with CA125 antigen followed by affinity reaction with a secondary antibody conjugated to gold nanoparticles (AuNP). With the addition of silver enhancement solution, metallic silver will deposit onto gold nanoparticles. The deposited metal was electrochemically stripped into solution and then measured by anodic stripping voltammetry (ASV). The stripping current signal reflects the amount of target protein, achieving a linearly relationship in the range from 0 to 50 U/ mL with a detection limit of 2 U/ mL human CA125 protein. The selectivity and reproducibility of the immunosensor were also evaluated.

PULSED ELECTRODEPOSITION OF NICKEL/PALLADIUM BINARY CODEPOSIT FROM GLUCONATE ALKALINE BATH. AN ELECTROCHEMICAL, XPS AND SEM INVESTIGATION.

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Pulse electrodeposition technique has been found to be a powerful means for controlling the electrocrystallization process and hence producing deposits with unique structures, physical and chemical properties. Our scientific interest is focused on the preparation of active composite and/or alloys electrocatalysts associated to the development of new electrode materials as sensing devices in electroanalysis. In particular, Pd-Ni electrocatalyst possesses many potential desirable features in terms of catalytic activities and electrochemical stability for many of these technological applications (1-5). In a continuation of our investigations, here we consider an efficient pulsed electrodeposition procedure for the preparation of a highly dispersed binary Ni-Pd film from a strong alkaline medium using a stable and nontoxic complexing gluconate electrolyte. The effects of several experimental conditions such as pulse waveform, time of electrodeposition, gluconate/hydroxyl concentration ratio, etc. on the kinetics of electrodeposition and film morphology are considered and critically evaluated. Thus, the X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) and electrochemical techniques were employed in order to ascertain the chemical state, composition, morphology and electrocatalytic activities of the relevant electrodeposited films.

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A NEW OFET DEVICE CONFIGURATION FOR HIGHLY PERFORMING BIO-ELECTRONIC SENSORS

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Bio-systems interfaced to an electronic device is presently one of the most challenging research activity that has relevance not only for fundamental studies but also for the development of highly performing bio-sensors. In this presentation the full integration of bio-systems such as phospholipid bilayers or proteins into an organic field-effect transistor (OFET) structure is proposed. Strikingly, the results show that both the electronic properties and the bio-layer functionality are fully retained. The platform bench-tests involved phospholipids and bacteriorhodopsin integrating OFETs exposed to 1-5% anesthetic doses that reveal drug-induced membrane changes. This challenges the current anesthetic action model relying on the so far provided evidence that doses much higher than clinically relevant ones (2.4%) do not alter lipid bilayers structure, significantly. Furthermore, a streptavidin embedding OFET shows label-free biotin electronic detection at 10 part-per-trillion concentration level, reaching state-of-the-art fluorescent assay performances. Extensive explored control experiments show the detection is also highly specific.
These examples show how the proposed bio-electronic platform, besides resulting in extremely performing biosensors, can open to gather insights into biological relevant phenomena involving interfacial modifications that can be electronically detected.

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MULTIVARIATE STRATEGIES FOR SCREENING EVALUATION OF CHRONIC ALCOHOL ABUSE

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An important goal of clinical medicine and forensic toxicology is to identify appropriate biomarkers of ethanol consumption to objectively support the diagnosis of chronic excessive alcohol intake. Commonly, screening of large population sets are executed by inexpensive determination of indirect biomarkers. The present study proposes a multivariate strategy based on five indirect biomarkers (AST, ALT, γ-GT, MCV and CDT), capable of considerably enhancing their individual diagnostic efficiency, specificity and sensitivity. Blood samples were collected from 240 healthy non-alcohol abusers and other 183 subjects, classified as non-drinkers, social drinkers and active heavy drinkers. ROC curves were determined on original biomarkers and mathematical combinations of them to provide an evaluation of their diagnostic performances in terms of discrimination between healthy non-alcohol abusers and heavy drinkers. The results from these univariate approaches were compared with those of the UNEQ class modeling multivariate strategy. The outcomes show that the multivariate approach can noticeably improve the screening potential of indirect biomarkers in the evaluation of alcohol misuse, with respect to the univariate strategy, and can be easily introduced in the clinical routine work. Only a moderate percentage of subjects (10%÷20%, at a fixed 10% or 5% misrecognition rate of heavy drinkers) is requested to undergo more expensive and time-demanding confirmation procedures, with a consistent reduction of work and expenses. The improved capability of the multivariate evaluation makes the reappraisal of indirect biomarkers topical, in contrast with the recent trend of considering their use void of practical significance.

MULTIVARIATE CHEMICAL MAPS FROM μ-FTIR AND DESI-MS HYPERSPECTRAL DATA

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Many modern analytical equipments allow to record spectral information across a surface. Chemical maps of compounds of interest – spatially located within the sample area investigated – are usually re-constructed by selecting a single spectral feature regarded as a marker for the compound. Nonetheless, such a univariate approach considerably underutilises the complex information embodied in the spectra, which are usually composed by hundreds/thousands of variables characterised by peculiar inter-correlations. The present study shows how multivariate methods are suitable to account for the complete spectral – and spatial – information from the samples studied. An interactive exploratory approach, based on principal component analysis (PCA) is applied to hyperspectral data arising from two different analytical techniques and application fields: μ-FTIR mapping, for characterisation and localisation of painting compounds in paint cross-sections (1), and DESI-MS mapping of biopsied human tissues, for chemical characterisation and differentiation of tumour and normal tissues (2). After PCA, a brushing procedure is performed in order to understand the relationships between the PC space and the map space, connecting chemical and spatial information. In particular, the score plot allows a visual inspection of the pixel distribution in PC space. In the score plot, it is possible to visualise groupings that indicate similarities among pixels, on the basis of the information derived from the spectra, and which can be associated with the particular characteristics of the samples analysed. With the brushing procedure, pixels with similar chemical profiles can be manually selected from the score plot in order to identify correspondences between the groups of points in the PC score plot and particular regions of the map. Finally, a joint examination of the loading score plots allows chemical characterisation of each part of the map to be achieved (1, 2).

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ANALYTICAL CHARACTERIZATION OF THE OLEORESIN OF *COPAIFERA LANGSDORFFII DESF.* (*FABACEAE*)

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Aim of this work was to carry out an extensive characterization of the Oleoresin (OR) from Copaifera langsdorffii Desf. (Fabaceae), by thermogravimetric analysis (TGA), GC-MS profiling, and molecular mass determination. OR is used in the therapeutic management of several inflammatory affections, i.e. sore throat, urinary, gastric and pulmonary diseases, and to heal skin ulcers wounds. TGA was carried out under N2 from 50 °C to 650 °C by a Perkin Elmer TGA7 Thermogravimetric Analyser. The DTGA thermal curve shows three peaks at 134 °C (22,7%), 248 °C (49,4%), and 371 °C (27,9%), each of them indicative of three main classes of components. Steam distillation of OR gives a volatile fraction (22% of the total), wich when submitted to GC-MS analysis shows a set of sesquiterpene constituents, the main of which were α-bergamotene, α-himachalene, β-caryophillene, β-elemene, cyclosativene, β-selinene, and paraffins. The bulk of these constituents, on the basis of their boiling points and total percentage content, very likely corresponds to the DTGA first peak. The residue was then submitted to derivatization (MeOH/6N HCl) and extracted with n-exhane.The extract, exhamined by GC-MS, exhibited a series of labdanic and labdenoic structures, diterpenoic acids, and diterpenes bearing α-β conjugated dienes, i.e. copalic, pimaric, isopimaric, abietic, daniellic, lambertinic, giberellic acids, the sum of them accounting to a 45% of the OR, close to the amount of the components present in the second TGA fraction. The average molecular weight (Mw) of the post n-exhane residue (≈35% of the total OR, coincident with the value of the last TGA peak), was determined on the OR sample with a multi-angle laser light scattering photometer (MALS, Dawn DSP-F from Wyatt) in off-line batch mode. The solvent used was ethanol at room temperature, the OR sample concentrations ranged from 0.7 mg/ml to 1.5 mg/ml. The MALS data analysis was performed using a conventional Zimm plot (i.e. a double extrapolation to zero angle and to zero concentration). The average molecular mass value was 8835 ± 660 g/mol. The polysaccharidic nature of the polimer (a xiloglucanic structure1) was confirmed by the positive response to the Dubois reaction (phenol/H_2SO_4) specific for carbohydrates.

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DIRECT ANALASYS IN REAL TIME MASS SPECTROMETRY FOR THE NON-INVASIVE IDENTIFICATION OF CONSERVATION TREATMENTS OF THE DEAD SEA SCROLLS

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The Dead Sea Scrolls are considered one of the most important archaeological discoveries of the 20th century. Most of the scrolls were written on parchment, and were unintentionally mishandled for the first four decades after their discovery. Thus today their long term conservation is a challenge. The non-invasive investigation of the conservation treatments they were subjected to is a crucial step in order to undertake the best conservation strategies. In the first years after their discovery no special attention was given to their preservation: irreversible damage was caused by using adhesive tape for joining fragments, castor oil was lavishly spread on the fragments to enhance the reading, glycerol and other chemicals were used in order to preserve the scrolls.

This paper will present here a quick and direct method for the non-destructive identification of conservation treatments of parchment by use of direct analysis in real time (DART) ionization and high resolution time-of-flight mass spectrometry. In this study Castor oil and glycerol treatments were investigated in order to evaluate two different classes of conservation processes. Exact mass determination on small parchment samples treated with castor oil and glycerol were completed at different working temperatures, with the instrument capable of operating at room temperature for non-invasive analysis: the technique was able to identify both conservation treatments.

Due to sensitivity, simplicity and lack of sample preparation, the proposed analytical tool could help conservators in the challenging analysis of unknown conservation treatments in cultural heritage.

ICP-AES ANALYSIS OF BYZANTINE ANONYMOUS COPPER COINS FROM THE XI CENTURY AND COMPARISON WITH MICRO-EDXRF NON-DESTRUCTIVE ANALYSIS

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Thirty-three Byzantine anonymous copper coins of century XI ("folles") were investigated in order to determine their chemical composition both in the core and in the surface. The aim of this study was also the identification of correlations of coin composition in coins from different coinage periods.

Inductively coupled plasma atomic emission spectroscopy (ICP-AES), a destructive technique, was employed for determination of major and minor metals (Cu, and Pb, As, Fe, Zn, Ag and Ni, respectively).

Sampling was carried out by mechanical drilling (1) using a tungsten-carbide drill (1 mm diameter; 20,000 rpm). Drilling started at the edge and extended on a radius to a depth of about 0.5 mm (Surface sample: S) and, in a second step, to a depth of about 3 mm (Core sample: C). Samples were weighted, dissolved in aqua regia and analyzed by ICP-AES. Folles average copper weight concentration was 92±3% for C-samples and 86±4% for S-samples.

For each coin we calculated the ratio between the minor element and the copper concentration: some coins showed significantly different Pb/Cu and Zn/Cu ratios, depending on their coinage period.

Micro-Energy Dispersive X-ray Fluorescence Spectrometry (micro-EDXRF), a non-destructive technique, was applied to analyze the same elements on the surface of coins (2). This technique is useful to gather information relative to surface composition since only low depths can be reached. We obtained very good correlations between Pb, Ag and As data from micro-EDXRF and S-data from ICP-AES.

Pb/Cu and Zn/Cu ratios are very useful to discriminate between different coins. We suggest micro-EDXRF as a rapid and non-destructive technique for the classification of these antique coins and in general for studies of historical samples (3).

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A HS-SPME-GCMS STUDY OF ROMANIAN AND BALTIC AMBER

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Amber is generated by fossilization of plant resin occurring in millions of years. This precious gemstone – which can assume different colours, ranging from pale yellow to reddish brown - is appreciated since ancient times and has been used for amulets and ornaments frequently found in archaeological excavations. Various analytical techniques, such as (Pyrolysis) - Gas Chromatography Mass Spectrometry (Py-GCMS) (1-3), Fourier Transform Infrared Spectroscopy (4,5), Raman spectroscopy (6), and thermal analyses (7), have been applied to characterize ambers from different origin.

In this study, Head Space-Solid Phase Micro Extraction (HS-SPME) GCMS was used to analyze the volatile fraction of Romanian and Baltic ambers. Although the chemical compositions of these ambers are very similar, some paleobiological and/or diagenetic differences have been evidenced (2,3,5). In this research it was found that differentiation can be accomplished by taking into account relative amounts of some volatile compounds and specific markers could be evidenced for both types of amber. These results show that HS-SPME-GCMS is a suitable non-destructive technique for analysis of fossil resins and may be applied to track the origin of archaeological amber findings.

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CHARACTERIZATION OF TRACE ELEMENTAL COMPOSITION IN PM10 SAMPLES MONITORED IN THE CITIES OF PIEDMONT REGION (ITALY)

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Atmospheric pollution resulting from airborne particulate matter, especially PM10 fraction, continues to be a major problem despite remarkable improvements having been made in terms of air quality over the last decades. Nowadays it has become very important to know the elemental composition and the sources of the airborne particulate matter in order to identify possible emergency situations in the environment resulting from bad air quality and consequently take action and implement recovery plans specific for the problems encountered.(1,2,3)

In this study we determined the concentration of the following elements: As, Ba, Cd, Co, Cr, Cu, Fe, Hg, K, Mn, Mo, Ni, Pb, Pt, Se, Si, Ti, V, Zn e Zr in airborne PM10 samples collected in Piedmont region: in particular, in two sampling sites in Turin (one located in the historical center of the town, the other on the northern outskirts of the town) and one in Biella. The samples were collected in different months in 2007. The analytes concentrations were determined using ICP-OES and ICP-MS.

Before the analysis of the real samples, an optimization of the procedure was made analyzing two certified materials, BCR 176 and NIST SRM 1649a.

The application of multivariate chemometric techniques (Principal Component Analysis and Hierarchical Cluster Analysis) to the experimental results allowed us to identify correlations among the investigated elements and to reveal similarities and differences between sampling sites, highlighting the existence of the main emitting sources as vehicular traffic/fossil fuel combustion and soil dust.

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MERCURY ISOTOPE RATIOS AS CONTAMINATION MARKERS: PROCEDURE DEVELOPMENT AND APPLICATIONS

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Mercury is one of the most harmful elements present in the Earth, and has both natural and anthropological sources. Moreover, Hg can undergo to many different transformation pathways during its biogeochemical, or industrial, cycles which in general involve redox reaction, both abiotic and biotic, and phase changes (1). Despite the toxicity of this pollutant, there is still a lack in the knowledge about the biogeochemistry of mercury in the ecosystem and, therefore, it is of utmost relevance to develop new scientific approaches to understand its transformation mechanisms and to identify its contamination sources. In this context, the determination of mercury stable isotopes ratios and, in particular, the identification of fractionation processes seems to be an extremely interesting and challenging application to verify the “provenance” of the element. Mercury, in fact, undergoes to both mass dependant, MDF, and mass independent fractionation, MIF, processes. In particular the MIF, involving only the odd isotopes (^{199}Hg and ^{201}Hg), appears to be a characteristic fingerprint of the process and the pathways involved in the Hg transformations (2). Thus, the study of both fractionation phenomena can be a powerful tool to identify its natural or anthropogenic source. This approach can be useful in case of polluted areas where many are the contamination sources in order to plan an environmental requalification. An intriguing case study is represented from the National Interest Site of the lagoon of Marano-Grado (Trieste, Italy), which is object of the present study. For these purposes, the evaluation of the isotopic composition in samples coming from this area has been performed by means of an HR-MC-ICP/MS system for the simultaneous determination of all the isotopes of interest (3). Due to the difficulties of the mercury ICP determination and the high number of the acquisition parameters the optimization and the validation of the analytical procedure was required, in order to obtain highly accurate and precise data. After this first step the method has been applied for the determination of the Hg isotopic fingerprints in environmental samples (e.g. sediments) coming from the Marano-Grado area.

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DETERMINATION OF WATER CONTENT IN ATMOSPHERIC PARTICULATE MATTER

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It is well known (1,2) that water is able to interact with organic and inorganic hygroscopic compounds of atmospheric particulate matter (PM) and that this interaction may alter most of the chemical and physical behavior of PM (solid-vapor equilibria, aerodynamic properties etc.).

The quantitative determination of water in PM has been attempted only in a few studies, mostly by using and indirect measurements based on the volume variation of the suspended particles before and after exposing the air flow to controlled relative humidity conditions (3).

In this work we report the optimization and validation of a new simple method for the quantitative determination of water in sampled PM and its first application to a series of real PM_{10} samples. The analyses are performed by a coulometric Karl-Fisher system equipped with a controlled heating device. Different water contributes are separated by the application of a proper thermal ramp. The optimal heating condition allows to distinguish several different types of water. The analytical performance of the method have been verified by using different standard materials. The recovery is always greater than 97% and detection limits are of ca. 20 µg. A sufficiently good repeatability (ca. 10%) is obtained both on reference materials and real PM_{10} samples.

The application of the method to real PM_{10} samples has evidenced that the amount of water is subjected to very relevant variation, as a function of the PM chemical composition. Mass percentages of ca. 3-4 % have been obtained in most of the samples, but values up to about 15% have been reached when the chemical composition of PM was dominated by secondary inorganic ions and organic matter. Very different thermal profiles have been also evidenced. To try an identification of the type of the released water, the method has also been applied to some hygroscopic compounds (pure SiO_2, Al_2O_3, NaCl, Na_2CO_3, ammonium salts and carbohydrates) that are likely present in PM.

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DETERMINATION OF Cd, Pb AND Cu IN SPRING WATERS OF THE SIBYLLINE MOUNTAINS NATIONAL PARK (CENTRAL ITALY) BY SQUARE WAVE ANODIC STRIPPING VOLTAMMETRY

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The general interest in heavy metal contents in spring and mineral waters refers to their potential toxicity and their compliance with national and international limits for maximum allowable concentrations. In the past only two attempts have been carried out to use a voltammetric technique (DPASV) for heavy metal determinations in mineral waters (1, 2). In this work we set up square wave anodic stripping voltammetry for the determination of Cd, Pb and Cu in spring waters using a method slightly modified from that applied by us in seawater (3). The work focuses on the spring waters of the Sibylline Mountains National Park (Central Italy) which in the past received attention only for bottled waters, both in European (4, 5) and Italian (6) studies. Samples were collected from three areas of the Park (Mount *Bove* North, Mount *Bove* South and Springs of River *Nera*) during the period 2004-2011. Besides metals some major ion concentrations and other physical-chemical parameters were also measured. Very low metal concentrations were observed (i.e., Cd 1.3±0.4 ng L^{-1}, Pb 13.8±5.6 ng L^{-1}, Cu 157±95 ng L^{-1}), well below the legal limits and also below the medians of known Italian (6) and European (4) data. Comparing the three areas it was noted that waters from the area of the *Nera* Springs are the poorest in heavy metals and the richest in minerals, that conversely the waters of Mt. *Bove* North are the richest in heavy metals and the poorest in mineral salts, and finally that intermediate values both for heavy metals and mineral salts were observed for the waters of Mt. *Bove* South. With very few exceptions, both mineral waters bottled in the area and aqueduct waters from public fountains show approximately the same metal contents as the spring waters from which they derive. Conversely some substantial metal increments are observed for sites of private houses which may be due to the presence of old metal pipes that release metals into the water.

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STUDY OF HUMAN FOSSIL BONES FROM AN ARCHAEOLOGICAL SITE OF MIDDLE NILE BY TG, DTG AND ICP SPECTROSCOPY.

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In this communication, results obtained from the thermal analysis of fossil bones coming from an important archaeological site (the necropolis of El Geili, in the middle Nile) are reported. TG-DTG analysis was carried out on several samples and the main steps in the thermogravimetric curves were characterized. First TG-DTG step "a" is connected to the loss of moisture; step "b", due to collagen decomposition, includes sometimes two DTG peaks, while other times only one; further TG-DTG steps at higher temperatures are related with carbonates decomposition. Using the main thermal data it was possible to assembly a table of numerical data, suitable to be processed by chemometrics. The PCA representation evidenced a good separation of all the studied samples in two different clusters. On the basis of studies published by G. Szoor (1), probably the separation of samples in two clusters can be attributed to different age of bones from different burials. However other researchers hypothesized that other reasons could be responsible of the differences found in the TG-DTG curves and consequently of the separation of the analysed samples in two different clusters, as was well evidenced by the chemometric representation. Therefore a deeper investigation was carried out, particularly focusing on the differences in the way collagen thermally decomposes, considering the different explanatory hypotheses reported in literature and experimentally studying the thermal decomposition of two types of pure collagen standards available on the market (observing both DTG peak temperatures and calculating the activation energy (Ea) values of collagen decomposition thermal breakdown). Further important observations derived from a detailed study of carbonate decomposition steps, considering separately the "secondary carbonate" and the "original carbonate" contained in the hydroxyapatite lattice. All these investigations seem to confirm and validate the idea that different age is probably the main cause of the separation of the examined samples in two different clusters. Lastly, the content of calcium, zinc and strontium was determined on the same samples by ICP emission spectroscopy and the obtained results were discussed on the basis of anthropologic models proposed in literature.

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STIR BAR SORPTIVE EXTRACTION AND LIQUID CHROMATOGRAPHY - TANDEM MASS SPECTROMETRY: A RAPID METHOD FOR TRACE ANALYSIS OF UV FILTERS IN DIFFERENT WATER MATRICES.

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Among emerging pollutants, organic UV filters have gained special attentions in monitoring aquatic ecosystems owing to the discovery of their ability to interact with human estrogenic receptors and thus interfering with the endocrine system (1). UV filters are substances that can filter UV radiation from sunlight and for this reason they are integrated in sunscreen creams for the protection of the skin. Moreover, they are employed as additives in cosmetics for daily usage (beauty creams, hair sprays, shower gels) and in products such as plastics, clothing or varnishes (2); therefore they are released into the environment by numerous ways. Humans can be exposed to UV filters through drinking water, seafood consumption, recreational activities, or absorption from the skin.

A new method using the extraction and preconcentration capabilities of stir bar sorptive extraction (SBSE), combined with fast liquid chromatography and tandem mass spectrometry was developed and applied to the determination of six UV filters in different water matrices.

Two ionization sources, atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI), were optimized and compared. APCI provided better results than ESI for all the analytes, with higher reproducibility and lower detection limits (3). Quantitative analysis was performed in multiple reaction monitoring (MRM) mode; calibration curves were drawn using SBSE in spiked water. A “data-dependent” acquisition mode (triggered MRM), was also used to increase throughput providing both quantitative and qualitative information in a single injection.

All figures of merit of the method were satisfactory; limits of detection (LODs) were particularly low for four out of six analytes ionized which resulted in the low ng/L range.

The method was applied to the determination of the UV filters in seawater, river water and wastewater samples collected in different sites of Liguria; results will be presented and discussed.

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TOTAL INTRODUCTION OF MICROSAMPLES IN INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY BY HIGH-TEMPERATURE EVAPORATION CHAMBER WITH A SHEATHING GAS STREAM

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There is an increasing interest in the elemental analysis of microsamples by inductively coupled plasma (ICP) spectrometry, including the analysis of limited-size samples (e.g. forensic, clinical, nuclear applications), the coupling of ICP spectrometry with low-flow separation systems (capillary electro-phoresis, micro-HPLC, microchip devices) and the analysis of specimens difficult to access (e.g. Antarctic snow) or scarcely tolerated by the ICP source (e.g. petroleum products). In order to reduce the sample consumption rate while keeping high the sensitivity, the application of a high-efficiency sample introduction system is mandatory. Recently, we developed a new total microsample consumption system, named TISIS ("Torch Integrated Sample Introduction System"), which provided superior performances in ICP atomic emission spectrometry over the conventional devices in terms of sensitivity, limits of detection, non-spectroscopic interferences and washing times.

In this follow-up study, a systematic investigation on the high-temperature TISIS for use in ICP mass spectrometry has been performed. The research included the optimization of the relevant parameters (chamber temperature, sheathing gas flow rate, nebulizer gas flow rate, sample uptake rate), the evaluation of its performance characteristics and representative applications to environmental, biological and clinical samples. Under the optimal conditions, the sensitivity was from 2 to 8 times higher than that measured using a conventional micronebulizer/mini-spray chamber system, due to the enhanced analyte mass transport towards the plasma and the solvent introduction in the vapour form. Short-term and long-term precision was better than 5%. Spectral interferences arising from common matrices were efficiently removed by the dynamic reaction cell technique and non-spectral matrix effects were comparable to those observed using conventional systems.

The application of TISIS/ICP-MS to representative certified reference samples (spinach leaves, marine plankton, bone tissue, human blood) proved the suitability of this system for the accurate analysis of limited-size samples.

A STUDY ON ALKYD PAINT MEDIA BY GC/MS AND HPLC-ESI-Q/TOFMS

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Alkyd resins were introduced as paint binders in art in the 1940s, and are an industrial evolution of the classical oil paint media. The adoption of these oil-based industrial polymers in art by painters as Frank Stella, Jackson Pollock and Pablo Picasso represents one of the milestones of the evolution of painting techniques: traditional natural binders as proteinaceous media and drying oils were replaced by a variety of organic synthetic paint materials, which dominated the XX century art scene.

Chemically, alkyds are oil-modified polyesters manufactured from polyols (typically glycerol or pentaerythritol), polybasic acids (phtalic anhydride, phtalic acid and its isomers) and a source of fatty acids, usually a vegetable oil.

In the context of the PAR-FAS Regione Toscana COPAC Project (*Preventive Conservation of Contemporary Art*, 2011-2013), we are investigating the chemical composition and the curing/ageing processes of alkyd paints.

The purpose of the study is to set up and apply advanced analytical procedures able to characterize the oils used for the alkyd resins production and to identify them in a paint sample. Moreover, we aim at assessing the conservation state and the entity of triglycerides oxidation in alkyd paint layers. This will contribute to deepen the knowledge of artworks painted with this technique and to improve conservation strategies.

We used GC/MS and HPLC-ESI-MS to study the triglyceride fraction of alkyd resins from different manufacturers (Ferrario and Griffin, Windsor & Newton), also subjected to artificial ageing. In particular, GC/MS analysis after hydrolysis and silylation allowed us to identify the fatty acid profile and the aromatic fraction of the paint material, and to study molecular changes associated to curing and ageing, e.g. oxidation of double bonds. This approach does not identify actual triglycerides (TAG) molecular species, but only determines the relative percentages of individual fatty acids. The use of modified oils, addiction of free fatty acids and additives in alkyd resins complicate the interpretation of the analytical results obtained by this technique. Thus, in order to obtain information on the TAG distribution, alkyd paints and reference oils were also analyzed by HPLC-ESI-Q/ToF-MS using positive-ion ionization and tandem mass spectrometry. This approach allowed us to identify TAGs and diglycerides in the material.

The results permitted to highlight the differences in the formulation of different kinds of commercial alkyd products, and to model the main reactions occurring during the curing of the investigated paint films.

AEROSOL CHARACTERIZATION BY PMF ANALYSIS OF SINGLE PARTICLE ATOFMS SPECTRA

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Aerosol time of flight mass spectrometry (ATOFMS) is one of the most powerful techniques which allows both size and chemical characterization of single airborne particles (1). Data analysis is still a challenge and in the present study, for the first time, PMF (positive matrix factorization) analysis was directly applied to single particle ATOFMS mass spectra, as opposed to data previously clustered by other techniques.

The analysis was performed on a total of 56898 single particle mass spectra, collected in Harwell (UK), allowing the extraction of 10 factors representing inorganic species, i.e. NIT (nitrate), SUL (sulphate), NaCl, and different elemental and organic carbon families including fresh EC, aged EC, oxidized organic aerosol, aromatic, and two organic nitrogen factors (2). In fact, the results show that PMF analysis applied to single particles makes a deconvolution of their mass spectra and it extracts factors with very well defined and characterized chemical profiles. In addition, for each extracted component (PMF factor), its time-series (both in terms of scores, equivalent number of particles and volume) is obtainable concurrently with its size distribution.

The results of PMF analysis were compared to those obtained from K-means cluster analysis and ART-2a artificial neural network analysis. Moreover, time-series of factors were compared with independent ion and non refractory organic carbon measurements and PMF-AMS factors (3) in order to evaluate the performance of the data analysis. The results showed that the time-series of PMF factors are correlated to the corresponding species concentrations and thus PMF analysis could prove to be useful also for quantification purposes.

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DEVELOPMENT OF A PORTABLE DEVICE FOR THE IDENTIFICATION OF OVALBUMIN IN PAINTING SAMPLES BY CHEMILUMINESCENT IMMUNOCHEMICAL CONTACT IMAGING

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The characterization of painting materials is fundamental for studying painting techniques and for restoration purposes. Among the various painting components, proteinaceous materials are of particular relevance because they are widely used as binders and adhesives. Immunological methods represent a powerful approach to protein identification, alternative to conventional chromatographic- and proteomic-based techniques. Moreover, immunoassays require simple instrumentation and can be performed using portable analytical devices, thus enabling on-site analyses. We have developed a portable ultrasensitive luminescence CCD-based biosensing device, in which the CCD is placed in direct contact with the sample to be analyzed ("contact imaging"). By using this device, we performed the immunochemical detection of ovalbumin (white egg chicken albumin), a protein found in egg tempera and in egg-based protective varnishes. The assay involved a simple extraction of the protein from paint samples followed by its detection by a noncompetitive immunoassay with chemiluminescent (CL) detection employing ready-to-use analytical cartridges. The target protein was captured by specific primary antibodies immobilized on a glass surface, then revealed by CL contact imaging using enzyme-labelled secondary antibodies and a suitable enzyme CL substrate. The assay was very fast and simple, and the protein could be identified with high sensitivity even in micro painting samples (0.5-2 mg). By using this portable device the analysis can be performed directly where the sample is obtained (point of need), without specialized personnel, thus reducing time and costs of the analysis. The method was validated by analysis of standard painting samples and comparison with reference MALDI mass spectrometry techniques. In perspective, the device could be also employed for the detection of other protinaceous components and organic compounds.

STRONG COMPLEXATION OF LEAD(II) BY FULVIC SUBSTANCES UNDER ENVIRONMENTAL RELEVANT CONDITIONS

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Because of the high concern of lead(II) in the environment, its speciation had been largely investigated in the past years. Different ligands of lead(II) can be present in natural waters ranging from inorganic anions, often at high concentration (chloride, carbonate) with side reaction coefficient around 30 (1), to very strong complexing sites present in NOM (natural organic matter). Side reaction coefficients of lead(II) slightly higher than those for inorganic ligands have been reported for humic substances (2). These sites are at low concentration but display a high complexation strength. Consequently they will be the first to take up the metal, while the weaker sites contribute to complexation only at higher total metal concentrations. Stronger lead(II) ligands have been detected in natural waters using a method with detection window much higher than the usual ones, based on the partition of the metal ion on complexing resins (3). Side reaction coefficients as high as about 10^6 were evaluated in natural waters.

A similar method (4) was here used to determine the complexing properties for lead(II) of a fulvic acid extracted from a sediment (FA), at low concentration, 10-500 nM. It is based on the sorption of lead(II) on the ion-exchange complexing resins Chelex 100 and Amberlite CG 50.

In the FA at pH around neutrality strong complexation sites of lead(II) were detected, with concentration 2 10^{-3} mmol g^{-1}-0.04 mmol g^{-1} similar to that determined by other methods (2), but conditional complexation constant as high as log K=13-15, much higher than that previously obtained, but similar to those found in natural waters (3). These sites heavily determine the lead(II) complexation since the metal in natural waters is present at nM level. A large influence of the solution pH on the complexing properties of the strong ligands has been found. On the other hand, using an ion exchange resin (Dowex 50W-X8) with lower detection window, lead(II) ligands with lower side reaction coefficient, near to that determined in previous investigations (2), were detected.

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INNOVATIVE UHPLC-MS/MS STRATEGIES FOR THE DETECTION OF DRUGS OF ABUSE, PHARMACEUTICAL DRUGS AND METABOLITES IN FORENSIC INVESTIGATIONS

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Several matters of forensic investigations involve the need of toxicological analysis, including lethal intoxication (suicide or homicide), environmental and workplace testing, abuse of pharmaceutical and/or illicit drugs as well withdrawal control, driving impairment or re-licensing, drug facilitated sexual assault, post-mortem toxicology, pre-natal exposure to drugs, doping control (1,2). The most utilized specimen are urine, blood and hair, each one having its own peculiar meaning, diagnostic window and analytical approach. To meet the high demand for drug screening in biological samples, toxicology laboratories are continuously encouraged to update their procedures, in order to target an increasing number of drugs but also achieve rapid, simple and sensitive analyses with reduced sample preparation and fast instrumental processing, so as to increase the overall sample-throughput. Our group recently developed and fully validated two screening procedures which take advantage from the last development of UHPLC-MS/MS technology. The first method is a sensitive multi-class and multiresidual screening method for detecting drugs of abuse or metabolites in hair samples using a dedicated UHPLC-MS/MS protocol. The second UHPLC-MS/MS method achieves the determination of 88 pharmaceutical drugs and metabolites in (post-mortem) blood samples, including the substances most frequently involved in acute intoxications and authoptic reports. For both methods, the analytical performances were highly satisfactory and relatively uniform for all the studied analytes, so that the protocols could find easy application in routine analysis for toxicological investigations. Some real cases will be presented.

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WHOLE-CELL BIOLUMINESCENT BIOSENSORS: A NEW WEAPON IN THE FIGHT AGAINST DOPING

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Many areas, such as medical diagnostics and anti-doping analysis, would benefit from devices that can perform a rapid and cost-effective screening without the need for equipped laboratories. Thanks to their ability to exploit highly specific biomolecular recognition mechanisms integrated within the detection system, biosensors can satisfy many of the analytical requirements related to on-site analysis. As a branch of biosensors, engineered bioluminescent (BL) cells exploiting BL reporter gene technology are now emerging as sensitive analytical tools.

Conventional testosterone doping tests are based on the urinary testosterone/epitestosterone glucuronides ratio (T/E) determination, usually performed by GC/MS.

We investigated weather a BL androgen-responsive yeast strain could be used as a rapid cost-effective anti-doping screening tool. Cells were genetically engineered to express the human androgen receptor (hAR) which drives the expression of *P. pyralis* wild-type luciferase through the regulation of the androgen responsive element (ARE) in presence of hAR agonists; an internal viability control relying on constitutive expression of the *P. pyralis* red-emitting mutant thermostable luciferase has been also introduced. Plasma and urine of healthy volunteers who were given 360 mg of testosterone i.m. were analyzed with the whole-cell biosensor. Briefly, cells were incubated with the sample in solution for 2 h at 30°C in 96-well microtiter plate format, then 50 µL of 1 mM D-luciferin were automatically injected and luminescence measurements (1 s integration) with high transmission band-pass filters were performed with Varioskan Flash reader luminometer. AR activity increased 4-5 fold two and four days after testosterone intake ($p<0.0001$), was back to basal activity on day 15 and, differently from GC-MS tests, was independent on the genotype. Other anabolic steroids and illicit drugs seized by State Police in Emilia Romagna Region were analyzed using a portable device, relying on a microwell cartridge in contact with a CCD sensor through a fiber optic taper. The assay showed more precise (intra- and inter-assay CV% 8 and 12%, respectively) and faster (total analysis time: 2 hrs) when compared to previously published cell-based assays, thus showing suitable for in-competition tests.

NEUTRAL LOSS AND PRECURSOR ION SCAN FOR THE SCREENING OF METYLENEDIOXYAMPHETAMINE- AND PIPERAZINE-DERIVED DESIGNER DRUGS IN URINE BY LC-MS/MS

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The consumption of psychoactive substances is an important social concern which is changing in the last years because of the introduction of several new substances (1). For example there are many possible amphetamine analogues, such as methylendioxyamphetamines, that, modifying the basic amphetamine structure, maintain its stimulant effect. Piperazines, cathinones and synthetic cannabinoids are other examples of designer drugs that represent an ongoing difficulty for analytical toxicologists since most of them are not detected by established analytical methods and immunochemical screening approaches are not always successful (2).

Liquid chromatography (LC) or gas chromatography (GC) coupled with mass spectrometry (MS) can be used for the screening of several compounds. GC-MS is the reference method and LC-MS(-MS) applications are still rather limited. Multi reaction monitoring (MRM) or single ion monitoring (SIM) have been used to screen a wide range of new drugs (3) but these procedures never constitute ''general unknown'' screening because of the preselection of the analytes. For this reason other acquisition modes in MS^2 as precursor ion or neutral loss scan appear very interesting.

This study describes a method for the screening and semi-quantification of metylenedioxyamphetamine- and piperazine-derived compounds in urine by LC-MS/MS. These substances, characterized by possessing common moieties, are screened using precursor ion and neutral loss scan mode and then quantified in MRM acquisition mode. Characteristic neutral losses and product ions were selected on the basis of the product-ion spectra (PIS) of known molecules belonging to the selected classes. The applicability of the screening approach was studied in blank urine, spiked with selected analytes and processed by SPE. Linearity, matrix effect, precision, accuracy, LODs and LOQs were evaluated both for the screening and the quantification methods, and the different results were compared. The ability of the screening method to provide semi-quantitative data was also demonstrated.

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IDENTIFICATION OF NEW DRUGS AND CREATION OF COMPOUND DATABASE FOR TIME OF FLY BASED TARGET SCREENING IN FORENSIC APPLICATION

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The use of liquid chromatography coupled with mass spectrometry offers several opportunities for analysis in forensic chemistry.
The ultra high pressure liquid chromatography (UHPLC) coupled to a high resolution mass spectrometer time of flight (HR Tof-MS) makes it possible to analyze samples with a relatively short pre-treatment and get results both qualitative and quantitative with a lower time of analysis, compared to the classic techniques such as gas chromatography.

Classical and new drugs like synthetic cannabinoids and cathinones, that constantly appear on the Italian market, counterfeit illicit medicines and Explosives residues have been analyzed to create and improve a Tof MS database. This allow the identification of different substances using “Targeted screening” based on retention time, on the exact mass (MS) and fragment product ions (MS^E).
MS^E is a novel, patented mode of data acquisition for Waters® Xevo G2Tof MS that provide a simple, unbiased, parallel route to delivering exact mass molecular (MS) and product ions in a single analysis.
Four new synthetic cannabinoids, used as ingredients for smart drugs, have been identified, during a survey. The characterization of these compounds has been made by gas chromatography-mass spectrometry (GC-MS), UPLC-HR-TOF, and nuclear magnetic resonance (NMR), leading to the identification of WIN48098, not yet found, to our knowledge, as adulterant in smart drugs, and AM679, AM2233 and JWH-307 identified in Italy for the first time.

Through the use of a ESCi source which combines advantages of ESI and APCI can be studied molecules such as explosives whose analysis is difficult using other techniques.

SCIENCE AND CONSCIENCE ON THE COURT: THE DATING OF HANDWRITTEN DOCUMENTS

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The dating of documents has become a valuable tool for the detection of fraud[1]. Medical malpractice, altered wills, divorces, wrongful terminations, insurance fraud, copyrights, labor-management disputes, and legal malpractice are situations that require the dating of documents.

The problem of dating of handwritings is quite complicated, so that the USA federal law used to oblige the ink manufacturers to add to inks different markers every year. Unfortunately many inks came from abroad so that the control of this enforcement was too difficult and then approach was then abandoned. Nevertheless the demand for handwriting dating was more and more increasing, so that many simple methods have been proposed.

The most popular techniques are the so called solvent extraction techniques. None of these methods has been neither seriously validated nor accepted by the American Society for Testing and Materials. Many papers, and our experience too, demonstrate they to be unreliable. Nevertheless many self-styled experts do continue to use these methods, in spite of their precarious results, just with the aim of cashing the fee, so causing serious damages to the enforcement of civil and criminal law.

We are now proposing a new method, based on the reflectance IR spectroscopy. In order to simulate different ages, handwritten documents were heated to 105°C for different times. Heating does not completely reflects the effect of the time, but the same procedure can be used for writing of different ages, when available. Paper are then extracted and the solutions are dried on an aluminum plate, from which spectra are acquired. After MSC treatment, discarding of correlated and of weak absorbances, a new type of multivariate calibration has been performed, where the heating time was a function of a linear combination of absorbances. Best predictions we achieved was a linear combinations of 5 absorbances. Of course a calibration line and chosen absorbances do depend on the composition of the ink. We then developed a discriminant analysis on several inks heated for different times that is able to distinguish the brand of the ink prior of determining its age.

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TANDEM MASS SPECTROMETRY OF SULPHUR-CONTAINING GLYCOLIPIDS: A STEP FORWARD TOWARDS THE REGIOCHEMICAL ASSIGNMENT OF FATTY ACID ACYL CHAINS

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Collision induced dissociation tandem mass spectrometry (CID-MS/MS) of glycolipids with special interest to sulfoquinovosyldiacylglycerols (SQDGs) (1,2) was employed for a detailed study of their fragmentation, clarifying some controversial aspects of previous investigations on these compounds. Losses of neutral fatty acids from the acyl side chains (i.e. $[M-H-R_xCOOH]^-$, $x = 1,2$) were found to prevail over ketene losses or generation of long-chain fatty acid (FA) anions $[R_xCOO]^-$, $x = 1,2$). The chain length, degree of unsaturation and positional distribution of the FAs attached to the primary (sn_1) and secondary (sn_2) hydroxyl groups of the glycerol moiety were established for all SQDG species identified in a sample extract of spinach leaves. The systematically observed preferential loss of FAs from the sn_1 position of the glycerol backbone was exploited for the regiochemical assignment of the investigated species (3). The prevailing presence of a 16:0 (i.e., palmitic) acyl chain on the glycerol sn_2 position of SQDGs suggests a prokaryotic path as the main route for their biosynthesis in spinach leaves (4). We envision that the versatility of this CID MS/MS approach, with ability to establish the regiochemistry of the acyl chains of SQDGs, will enable the systematic investigation of photosynthetic plants, algae, cyanobacteria, purple sulfur and non-sulfur bacteria with broad implications within and beyond the realm of sulfolipids and their involvement in membrane structures and cell communication.

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NOVEL DIOXETANE-DOPED SILICA NANOPARTICLES AS ULTRASENSITIVE REAGENTLESS THERMOCHEMILUMINESCENT LABELS FOR BIOSENSING

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Luminescence detection is particularly attractive for bioanalytical applications and biosensors because it combines high detectability with simple instrumentation. Even if fluorescence, bio-chemiluminescence and electrogenerated chemiluminescence are the most common luminescence detection techniques, other techniques have been investigated over the years. Thermochemiluminescence (TCL), *i.e.*, the light emission originating from the thermolysis of a molecule, was proposed in the late '80s as a detection technique for immunoassays (1,2). However, after little pioneering work, TCL detection was abandoned due to methodological problems, such as the high temperature required to trigger the emission (200-250°C), and to the poorer detectability in comparison to other labels.
Herein, we report for the first time the TCL properties of an acridane-based 1,2-dioxetane showing a remarkably low (below 100°C) TCL triggering temperature and describe amine-functionalized TCL silica nanoparticles (SiNPs) incorporating this compound, either alone or together with a fluorescent energy acceptor, to be used as TCL labels in bioassays. Thanks to the signal amplification due to the high 1,2-dioxetane loading, the detectability of the doped SiNPs is comparable to that of enzyme labels. In addition, preliminary experiments showed that the doped SiNPs could be conjugated to antibodies maintaining their binding ability.
Therefore, these labels could pave the way for the revival of TCL detection in bioassays. Miniaturized, simple and ultrasensitive analytical devices and biosensors could be developed by taking advantage of the high signal/noise ratio of TCL detection and the possibility to perform the measurement without any addition of reagents.

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IDENTIFICATION OF GENES DYSREGULATION IN DIAMOND-BLACKFAN ANEMIA THROUGH GENOMICS AND MULTIVARIATE DATA ANALYSIS

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DBA is an autosomal dominant bone marrow failure syndrome due to a defect in genes encoding for ribosomal proteins. The patients show pure erythroid aplasia with normal bone marrow and peripheral blood counts of the other hemopoietic cell lines. The link between erythropoiesis and the ribosome is still somewhat obscure, although several lines of evidence suggest that an abnormal ribosome biogenesis switches on a condition named "ribosomal stress" leading to stabilization of p53, proliferation block and induction of apoptosis. However, also p53-independent pathways have been proposed.

To identify p53-negative pathways activated by ribosomal stress, we have analyzed global gene expression in erythroid cell lines carrying p53 mutations (TF1) that were silenced for RPS19, RPL5 or RPL11 and compared to their scramble counterparts using Affymetrix arrays.

Multivariate analysis through Ranking - Principal Component Analysis was applied on the identified datasets together with data obtained from fibroblasts of DBA patients to define an intersection that represents the common alteration of different RP deficiencies.

The analysis through Ranking-PCA allowed to observe the dysregulation of genes involved in protein synthesis, apoptosis, redox regulation. Increased ferritin and reduced superoxide dismutase 2 were also observed in CD34+ cells downregulated for RPS19. These data show that dysregulation of defined molecular functions and cellular processes represent a common feature of human cells with RP deficiency. The increased ferritin may be linked to an abnormal iron metabolism in these cells.

PROTEOMIC ANALYSIS OF PLATELET MICROPARTICLES BY NANO-HPLC/HIGH RESOLUTION MASS SPECTROMETRY

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Microparticles (MPs) are a heterogeneous vesicle population (100–1000 nm) virtually released by all eukaryotic cells in a highly controlled process triggered by various stimuli during cell activation, stress conditions, differentiation, senescence, apoptosis and upon cell damage (1). MP release is a fundamental capacity because it allows cells to selectively concentrate and release part of their content into the surrounding milieu(1), participating to the local and systemic intracellular communication by two mechanisms: MPs can behave as circulating messengers exposing membrane, bioactive molecules or they can act as vehicles and directly transfer part of their content, including proteins, RNA and bioactive lipids, inducing activation, phenotypic modifications or reprogramming in the target cell, both in physiologic and pathologic conditions. The presence of negatively charged phospholipids promotes the formation of procoagulant protein complexes contributing to haemostasis (2).The number of circulating MPs, cellular origin and composition vary according to type and state of a disease and medical treatment. Despite their biological roles, currently there is no standardized method for qualitative and quantitative analysis of MPs (3). We chose to apply a modern shotgun proteomics approach to provide a simplified and effective experimental procedure for their characterization to furnish the analytical tools for protein identification and the basis for understanding their roles in cell communication. Platelet MPs have been isolated from ADP-stimulated platelets by differential centrifugation and the extracted proteins split into two aliquots, then processed according to two different procedures: a standard shotgun proteomics procedure, in which proteins have been denaturated and in-solution digested, and a modified version, in which a fractionation step by hydrogel nanoparticles has been added to improve protein identification of potentially interesting low molecular weight proteins. Analysis bynanoHPLC-LTQ Orbitrap XL mass spectrometer system, Mascot database search and Scaffold validation provided a more simple and straightforward procedure with respect to previous described methodologies for the study of platelet MPs, producing a tool for further understanding their biological and pathological roles.

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BIOANALYTICAL ASSAYS FOR DNA-B[a]PDE ADDUCTS DETECTION

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Humans are exposed to complex mixtures of toxic chemicals like polycyclic aromatic hydrocarbons (PAHs) that must be strictly monitored because of their carcinogenic, mutagenic and teratogenic effects. Benzo(a)pyrene (BaP), the most widely studied and representative compound of this class of chemical carcinogens, exerts carcinogenic property after metabolic activation. Its main toxic metabolite is B[a]PDE, which binds to the exocyclic amino group of guanine in DNA to form a covalent adduct. Different approaches have been recently attempted by our group for the detection of this genotoxic compound (1, 2). Here we present two bioanalytical assays for B[a]PDE-DNA adducts detection based on a SPR (Surface Plasmon Resonance) DNA biosensor and RAPD (Random Amplified Polymorphic DNA) -PCR. The quantitative PCR assay is based on the ability of damaged DNA to inhibit DNA polymerases, thus interfering with replication of the template DNA and decreasing the yield of PCR product. Treatment of different genomic DNA (*Enterococcus Faecalis, Saccharomyces Cerevisiae* and *Lactobacillus Plantarum*) as well as different primers (M13: GAG GGT GGC GGT TCT; LA1: GCG ACG GTG TAC TAA C) resulted in different electrophoretic pattern of the amplified DNA. The SPR-based biosensors approach relied on the inhibition of the hybridization of selected oligonucleotides after formation of the DNA adduct. Quantitative information as well as difference in the kinetics has been observed and characterize for different oligonucleotides. These bioanalytical assays could be used for the detection of potential genotoxicity of different chemicals.

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METABOLOMICS OF TRANSGENIC PLANTS UNDER CHEMICAL STRESS

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Phyto-hormones play a key role in plant response to many different biotic and abiotic stresses since a modified hormonal profile up-regulates the activation of secondary metabolites involved in the response to stress. Thus, assessing the changes in the phytohormone profile and other metabolites of a biological system, i.e. *Nicotiana langsdorffii* genotypes normal and transgenic, after a controlled exposure to contaminated culture medium, is very important in terms of understanding the mechanisms and effects on the growth processes and development of transgenic plants and requires the optimization of reliable analytical procedures for the determination of selected chemical parameters. In this respect, a coordinate activity of several analytical chemistry groups and a genetic biology group allowed us to obtain *Nicotiana langsdorffii* normal and transgenic plants which were grown in controlled conditions and to determine abscisic and indole-acetic acid, along with salycilic and shichimic acid, total polyphenols, chlorogenic acid, antiradical activity and element distribution patterns. In the present work transgenic GR plants and isogenic wild type genotypes have been exposed to metal stress treating them with 30 ppm Cadmium(II) and 50 ppm Chromium(VI). Hormonal patterns along with the changes in key response related metabolites were then accurately monitored and compared. Both Cd and Cr treatments induced an increase in hormone concentrations and secondary metabolites only in wild type plants, whereas heavy metals absorption has been found to be lower in the case of GR plants. Moreover, metal exposure strongly affected accumulation of several elements. These result are finally discussed proposing that the response to stress due to changes in the plant hormonal system may derive from the interaction between the GR receptor and phytosteroids, known to play a key role in plant physiology and development.

COUPLING FLOW FIELD-FLOW FRACTIONATION WITH PHOTOLUMINESCENCE SPECTROSCOPY FOR THE CHARACTERIZATION OF MULTIFUNCTIONAL NANOMATERIALS

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Synthesis and applications of new functional nanoparticles are finding increasing interest in many fields of nano(bio)technology. Chemical modifications of inorganic nanoparticles are often necessary to improve their features as spectroscopic tracers or chemical sensors, and to increase water solubility and biocompatibility for applications in nano(bio)technology. Recent reports by institutions such as the FDA and the European Union acknowledge the lack of rugged analysis and characterization methods for nanomaterials as a major limiting factor to the final establishment of nanotechnologies.

Analysis and characterization of structured nanoparticles in fact are key steps for their synthesis optimization and final quality control. Today's most used functionalities in nanomaterials include fluorescent groups and drug molecules. In this work it is shown that asymmetrical flow field-flow fractionation online coupled with multi-angle light scattering and photoluminescence spectroscopy can become the elective methodology for size and optical characterization of multifunctional, fluorescent nanoparticles. This work shows that the approach based on these coupled techniques allows size fractionating the nanoparticles, and separating them from the unreacted fraction of the functional group. It is also possible to evaluate the level of coating of the tags used to functionalize the nanoparticle surface or the actual inclusion and self-organization of the molecules inside the nanoparticles, as well as to monitor possible aggregation of the nanoparticles or release of the surface functionalities.

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STRATEGY TO TUNE EXTEND AND NARROW THE DYNAMIC RANGE OF APTAMER-BASED SENSOR

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The high specificity and affinity binding of proteins and nucleic acids have inspired decades of research aimed at employing biomolecular recognition in novel diagnostic tools. Despite this enthusiasm, however, biological recognition elements often exhibit a potentially significant limitation: the single-site binding characteristic of the majority of such receptors produces a hyperbolic dose-response curve with a fixed dynamic range. This can limit the utility of biomolecular receptors in applications which require the measurement of large changes in target concentration or that require a strong sensitivity response (a steeper variation of output signal with small amount of target concentration).

We have previously demonstrated different strategies to tune, extend and narrow the dynamic range of classic DNA optical and electrochemical biosensors (1, 2). Here we follow-up on these previous works and demonstrate multiple, complementary approaches by which we can tune, extend and narrow the dynamic range of a model aptamer-based cocaine sensor. Specifically, using a mutational approach we have generated sets of cocaine aptamers varying in their affinity for the target. Using various combinations of these receptors we were able to both narrow and broaden the dynamic range of biochemical receptors. In a second approach we have used a model cocaine aptamer and have changed its affinity using allosteric effectors. Compared to the mutational approach, this method provides a more rational, more efficient, and more cost-effective approach by which we can tune the affinity of an oligonucleotide-based receptor. Moreover, we demonstrated that using different combinations of allosteric effectors we can extend the dynamic range of the aptamer up to 4 orders of magnitude.

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SURFACE ANALYTICAL CHARACTERIZATION OF MULTIFUNCTIONAL ZnO_X-FLUOROPOLYMER NANO-COATINGS FOR THE TEXTILE INDUSTRY

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Ion Beam co-Sputtering (IBS) of ZnO and polytetrafluoroethylene targets can be proficiently used for the production of novel coatings composed of ZnO nanoparticles (NPs), finely dispersed in a polymer matrix. Different ZnO-NP loadings (φ) are achievable by properly tuning the material deposition conditions. The resulting nanostructured coatings combine the ZnO-NP antimicrobial properties with the water repellence and anti-stain characters provided by the fluoropolymer dispersing matrix.

In this study, X-ray Photoelectron Spectroscopy (XPS) has been used to quantitatively asses the materials' surface chemical composition, as a function of φ. Six to seven carbon species could be detected in C1s spectra, the abundance of highly fluorinated moieties being inversely related to φ. Two peaks were detected in the F1s spectra, and they could be attributed to inorganic, as well as organic species. Same was observed in the case of O1s XP regions. Zinc surface chemical speciation could be addressed by the indirect quantification of zinc fluoride and oxide species (by curve-fitting the O1s and F1s XP spectra), as well as by the direct study of the Zn $L_3M_{45}M_{45}$ Auger signals. As a result, the surface NP composition was interpreted in terms of the simultaneous presence of ZnOx and of small amounts of ZnF_2 species. Fourier transform infrared spectroscopy was used to characterize the materials' bulk composition, while transmission electron microscopy was used to morphologically characterize the nanocoatings. The antimicrobial properties of the ZnO-containing products were successfully demonstrated on two target microorganisms: gram-positive *Staphylococcus aureus* ATCC 25923 and gram-negative *Escherichia coli* ATCC 25922.

The promising results strongly encourage the application of ZnOx/fluoropolymer nanoantimicrobial coatings in the textile industry.

GROWTH OF SULPHIDE THIN FILMS WITH TECHNOLOGICAL INTEREST

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In the last years research on renewable energy has become more important because of increasing of energy demand combined with the need of sustainability. In particular there is a big interest on photovoltaic energy and above all on thin film solar cell. Producing this kind of cell is cheaper compared to wafer-based solar cell. Current thin films solar cells are made of CdTe, a-Si or CuInxGa1-xS(Se)2 (CIGS) but these materials are low abundant on Earth's crust and they could give toxicity problems. New materials like kuramite (Cu3SnS4), kesterite (Cu3ZnSnS4) and stannite (Cu2FeSnS4)-type materials are promising semiconductors for solar cells because of their suitable optical band gap, abundance and low environmental impact compared to CIGS CdTe and a-Si. Semiconductors are generally prepared by high temperature solidification methods from the elements in bulk form, or vapor phase and vacuum methods in the form of thin films[1][2]. The growth of these materials in bulk form or as thin films is also possible from liquid solutions. For this reason electrodeposition techniques are emerging especially as methods for the synthesis of semiconductor thin films and nanostructures[3]. Electrodeposition could be applied on large scale thanks to its economy, low environmental impact (we can work with aqueous solution) and the low working temperature. The electrochemical method of deposition ECALD (Electrochemical Atomic Layer Deposition) resulted a valid approach to prepare semiconductor compounds on metallic substrates. This technique is based on the alternate underpotential deposition (UPD) of atomic layers of the elements constituting a compound in a cycle that can be repeated many times to obtain the desired thickness. We realized thin films of ternary compounds CuxSnySz with different thickness and composition with ECALD technique. The obtained films were characterized by AFM, XPS, stripping voltammetry and UV-vis spectroscopy. AFM measurements showed a similar morphology of the film compared to the substrate. From XPS analysis we observed that the stoichiometric Cu/Sn ratio changes if we perform different numbers of cycles of each metal, even if the ratio is whenever shifted to Cu. The band gap values of the film estimated with UV-vis experiments are suitable as semiconductors in solar cells.

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NANOSTRUCTURED ENZYMATIC BIOSENSOR BASED ON FULLERENE AND GOLD NANOPARTICLES: PREPARATION, CHACTERIZATION AND ANALYTICAL APPLICATIONS

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The direct electron transfer between redox proteins and the electrode surface in a third generation biosensor is often enhanced by using nanostructured electrochemical materials. To this aim, fullerene and gold nanoparticles (AuNPs), represent really promising compounds due to their remarkable electrochemical properties (1,2).

In this work a new approach for the fabrication of a nanostructured enzyme-based biosensor, that exploits the synergistic beneficial features of functionalized fullerene and AuNPs is proposed, in order to obtain a significant improvement of the electroanalytical properties of the device.

The biosensor was firstly realized by immobilizing functionalized AuNPs with mercapto-carboxylic acids of different length on a gold electrode surface, modified with a self assembled monolayer (SAM) of cysteamine; subsequently polyhydroxy-fullerene has been linked onto the modified electrode, where the enzyme has been finally immobilized. The influence of different modification step procedures on the electroanalytical performance of biosensors respectively based on *Trametes versicolor* Laccase (TvL) and *Horseradish* Hydrogen Peroxidase (HRP) has been evaluated.

Cyclic voltammetry, chronoamperometry, surface plasmon resonance and atomic force microscopy were used to characterize the modification of surface and to investigate the bioelectrocatalytic response of the biosensor.

A tentative application of the developed enzymatic electrode was performed evaluating the detection of some phenolic compounds by using enzymes above mentioned (3).

The proposed strategy increases the amount of electroactive protein on the electrode and also enhances the electron transfer between the redox center of the protein and the electrode surface, moreover it provides a new versatile and powerful platform for biosensor design and biological applications.

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ADVANCED DETECTION OF GENETIC DISORDERS BY SURFACE PLASMON RESONANCE IMAGING

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Most of the currently available nucleic acid detection methods require the amplification of the target species and detect the duplex formation by using labels and transducers able to generate signal as a consequence of the specific hybridization event. Both amplification and labelling processes are laborious, can create artefacts and may interfere with the hybridization reaction. In this perspective, the direct detection of non-amplified genomic DNA appears an excellent cost-effective alternative to the PCR-based approach, since extra labour and cost from the amplification procedure are reduced. Recently, we have shown (1) that an ultrasensitive detection of non-amplified genomic DNA containing a target sequence as a minor component can be obtained by using nanoparticle-enhanced Surface Plasmon Resonance Imaging (SPRI) and Peptide Nucleic Acids (PNA) probes. In this work (2) we describe the ultrasensitive nanoparticle-enhanced SPRI detection of SNPs in non amplified genomic DNAs carrying the mutated β°39-globin gene sequence. Attomolar concentrations of target genomic DNA have been detected and DNAs from healthy individuals and homozygous or heterozygous β-thalassemia patients have been discriminated. Our method required simple processing of the genetic samples. The reduced number of genomic DNA equivalents required for the analysis allows to propose our method as a potential new tool for the genetic diagnosis based on the analysis of circulating free fetal DNA in the blood of the pregnant woman.

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SPECTROSCOPIC CHARACTERIZATION OF A SOLID CATALYST FOR DIOXIRANE-MEDIATED HETEROGENEOUS EPOXIDATIONS

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The catalytic oxidation of alkenes is a process of continuing general interest. Some oxidation methods employ non-metal organic catalysts, such as ketones, which serve well to generating dioxirane intermediates, which are very efficient and remarkably versatile oxidants (1). In this respect, we synthesized and characterized a novel hybrid material that exhibited suitable catalytic performances, consisting of trifluoromethyl ketone (TFMK) moieties immobilized on silica via an appropriate spacer (2). This new TFMK catalyst presented obvious advantages over other similar catalytic materials (3), thanks to its higher stability and efficiency.

The new catalyst became fully characterized by a combination of FTIR, NMR, and XPS spectroscopic techniques. In particular, XPS has been used for the surface characterization of both the ketone catalyst as well as its precursor, i.e. the silica-CO_2H. Besides the expected F1s signal (688.3 eV) in the wide scan spectrum of the catalyst, the high-resolution C1s region presents new contributions at 286.6, 288.4, and 292.7 eV. Consistent with literature (4), the latter two components are assigned respectively to $C(:O)CF_3$ and to CF_3 groups. Useful hints concerning the fate of catalyst after exhaustive working could be gathered by quantitative ^{19}F NMR and XPS analysis. For instance, on going from the initial active catalyst to its almost exhausted form, the XPS C1s spectrum showed a significant drop of the carbonyl (288.4 eV) and CF_3 (292.7 eV) contributions.

In conclusion, XPS investigation has played a key role in providing relevant information on surface chemical composition of the catalyst, closely connected with its stability and performance.

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A NEW STRATEGY FOR PRESSED POWDER EYE SHADOWS ANALYSIS: ALLERGENIC METAL IONS CONTENT AND PARTICLE SIZE DISTRIBUTION OF THE INSOLUBLE MATTER

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Before being placed on the market, all cosmetics undergo a battery of safety tests to safeguard consumers from possible side-effects. Despite these controls, sometimes cosmetics do have side effects: some are immediate and visible reactions, others may appear with prolonged use.

Among decorative cosmetics, eye shadows deserve particular attention because they are applied in the peri-ocular area, the area around the eyes where the facial skin thinnest; here the risk of percutaneous absorption of the pigments — and thus of toxic elements — is very high as is the risk of developing irritative and/or allergic skin reactions.

In this work nine compact powder eye shadows — very inexpensive products sold in Italy and targeted to children and adults — were examined for the first time in order to i) determine the Ni, Co and Cr concentrations, ii) quantify the "water" soluble chromium and at the same time, iii) obtain the particle size distribution of the water-dispersible submicro-particles contained in all powders.

In many cases, the Cr, Co and Ni concentrations, determined by Graphite Furnace Atomic Absorption Spectrometry (GF-AAS), were higher than 1 or 5 ppm (µg/g), i.e. the limits recommended in the scientific literature to minimize the risk of reaction in particularly sensitive subjects. In most cases, the concentration of Cr was higher than that of Ni and Co, up to a limit case of 150 mg/g. In this particular sample, the potential amount of Cr that could be released in ionic form was determined in sweat simulating solutions by GF-AAS and confirmed through a specific spectrofluorimetric method; the results indicated the presence of approximately 80-90 ppb (ng/g) of Cr^{3+}.

The water dispersible particles were isolated from the eye shadow powders through a simple solvent extraction procedure. The aqueous suspensions were then sorted through Sedimentation Field Flow Fractionation (SdFFF) and the particles sizes were calculated from experimental fractograms using theory. For the most part, the computed sizes were in the micron range, as confirmed by some SEM photographs taken on fractions collected during the separations. The SdFFF coupled off-line with the GFAAS enabled elemental characterization of pigment particles as a function of size.This finding reduces the concern that the ingredients of such makeup formulations may contain nanoparticles.

NON-DESTRUCTIVE DEPTH PROFILE RECONSTRUCTION OF BIO-ENGINEERED SURFACES BY PARALLEL ANGLE RESOLVED X-RAY PHOTOELECTRON SPECTROSCOPY

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Label-free biosensors are of considerable interest for various clinical and biological applications. In these systems, achieving an optimized receptor immobilization strategy critically influence the sensing performance in terms of specificity, sensitivity, response kinetics and detection limits. However, monitoring the receptor spatial organization and the interfaces composition on a nanometer or sub-nanometer scale is a very hard challenge. In the present contribution Parallel Angle Resolved X-ray Photoelectron Spectroscopy (PAR-XPS) was proposed as useful tool to address the challenge of probing the near-surface region of bio-active sensors surface (1). A model receptor was chosen and a well-established functionalization procedure (2) was systematically characterized by PAR-XPS. Commercially available Thermo Avantage-ARProcess software was used to generate non-destructive concentration depth profiles of protein functionalized silicon oxide substrates. At each step of the functionalization procedure, the surface composition, the over layer thickness, the in-depth organization and the in-plane homogeneity were evaluated. Compared to multi-techniques characterization approaches previously proposed in the literature, the present analytical approach boasted the peculiar advantage of providing, simultaneously, morphological and compositional information from the same data set. The critical discussion of the generated profiles highlighted the relevance of the information provided by PAR-XPS technique.

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ARSENIC REMOVAL BY INTERACTION WITH GYPSUM: EFFECT OF PH, AS (V) CONCENTRATION AND PARTICLE SIZE.

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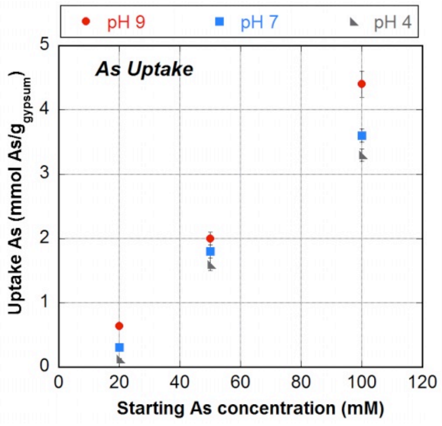
Arsenic-bearing sulfide minerals give rise to great environmental concern owing to the risk of toxic As release as effect of their oxidation/dissolution. [1]. According to the European regulations (e.g. Council Directive 98/83/EC) As content in drinking water has to be lower than 10 μg/dm^3. Arsenic can be removed from water by several methods [2] and in this work the immobilization of arsenate ion on gypsum ($CaSO_4*2H_2O$) was investigated. Gypsum was chosen for two main reasons: 1) its low cost, 2) it is often present in mine wastes together with arsenic minerals thus it might contribute at arsenic immobilization together with ferrihydrite [3]. Arsenic removal by gypsum was investigated by means of ICP-OES and AAS as a function of: arsenate concentration (20mM, 50 mM, 100mM), pH (4, 7, 9) and gypsum particle size (ranging between 40μm and 4800 μm). The removal was found to follow a pseudo-second order kinetic. Best results were obtained at pH = 9, starting with higher arsenic concentration and with gypsum particle size between 800 μm and 2000 μm. Due to the solubility of precipitated calcium arsenates the concentration of arsenic in the solutions was always higher than 10 μg/dm^3. Arsenic removal by gypsum might be used as a first, cheap, method to decrease arsenic content in highly contaminated water.



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APTAMERS IN BIOSENSORS: RECENT ADVANCES AND POSSIBLE APPLICATIONS

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DNA and RNA aptamers represent an interesting class of receptors in affinity-based biosensors (ABBs), alternative to antibodies. They are linear sequences, generally ranging from 15-60 bases in length, obtained by *in vitro* selection, i.e. by Systematic Evolution of Ligands by Exponential Enrichment (SELEX) approach. The ideal candidate should be able, in suitable conditions, to fold its primary sequence in a specific three-dimensional structure to bind the target molecule applied during the selection process. By SELEX, it is theoretically possible to select aptamers against any molecular target; at present, aptamers have been selected for small molecules, peptides, proteins, viruses, and bacteria. In this framework, aptamers find many applications in medicine as well in bioanalytical, for example biosensors (aptasensors) development. We present here some advances in aptasensors development and possible applications for clinical diagnostic and therapeutic protocols, as well as promising applications of aptasensors to the anti-doping field. Both DNA and RNA aptasensing has been developed versus protein of clinical interest, using both optical (Surface Plasmon Resonance and SPRi imaging) and piezoelectric sensing. The analytical parameters of the systems will be discussed.

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CHCA-BASED NEW MATRICES FOR MALDI-MS ANALYSIS OF LIPIDS AND PEPTIDES

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A significant area of study and upgrading for increasing sensitivity and general performance of MALDI is related to the matrix design.

Several efforts are also made to solve the problem of low-mass-region interference, especially for lipid analysis. Different matrix-free approaches involving laser desorption/ionization (LDI) of analytes [1] or the use of unconventional matrices as metal nanoparticles [2,3] have been successfully investigated in our laboratory. Alternatively, we proposed a number of new matrices as lumazine [4], ionic liquid [5] and proton sponge [6] for studying a wide range of analytes from amino acids to intact bacteria.

Recently, new rationally designed matrices as 4-chloro-α-cyanocinnamic acid (ClCCA) have been introduced and reported to provide improved analytical performances [7,8]. This matrix showed to be a superior alternative to the commonly used α-cyano-4-hydroxycinnamic acid (CHCA). We have taken this rational design one step further by developing and optimizing new MALDI matrices chemically similar to CHCA but with different functionalities and substituents. In particular, we were interested in understanding the effect of several electron withdrawing (e.g. nitro-) or donating (e.g. methoxy-) groups or the extent of conjugation on the ionization efficiency. Potential matrix molecules were designed on a rational basis and subsequently synthesized, purified using solid phase extraction (SPE) technique, characterized by NMR and UV spectroscopies and finally tested as matrix for lipids or peptides. Some of them displayed good to even excellent performance as MALDI matrices.

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SYNTHESIS AND CHARACTERIZATION OF TAILORED SURFACES AS SUPPORTS FOR DESORPTION ELECTROSPRAY IONIZATION-MASS SPECTROMETRIC INVESTIGATIONS

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So far a great number of papers have highlighted desorption electrospray ionization-mass spectrometry (DESI-MS) as a high-throughput technique in a variety of application fields. Several papers have been published elucidating the ionization mechanisms leading to the formation of isolated gas-phase ions. In this context, the role of surface and pneumatic effects on ion-formation yield has recently been investigated (1,2). Nevertheless the effect of the surface chemistry has not yet been completely elucidated. Functionalized glass surfaces have been prepared, in order to tailor surface performance for ion formation. Three substrates were functionalized by depositing three different silanes (3-mercaptopropyltriethoxysilane, MTS, octyltriethoxysilane, OTES and 1H,1H,2H,2H-perfluorooctyltriethoxysilane, PFOTS) from toluene solution onto standard glass slides. Surface characterization was carried out by contact angle measurements, tapping mode atomic force microscopy and X-ray photoelectron spectroscopy. Morphologically homogeneous and thickness-controlled films in the nm range were obtained, with surface free energies in the 15-70 mJ/m^2 range. These results will be discussed together with those of DESI-MS on low-molecular weight compounds such as melamine, lincomycin and tetracycline, also taking into account the effects of different spray solvents as well as temperature and capillary voltage.

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DEVELOPMENT OF AN IMPROVED PERFORMANCES PROTOTYPE CHAMBER FOR LASER ABLATION ICP-QMS

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Laser ablation - inductively coupled plasma - mass spectrometry (LA-ICP-MS) is a powerful method to determine trace elements in solid samples, as it combines the high sensitivity and isotope selectivity of ICP-MS detection and the efficiency of laser ablation sampling. The spatial resolution attainable with commercially available laser systems is particularly suitable for applications such as elemental mapping or in-depth profiling. While both lasers optics and ICP-MS instruments have reached nowadays a very good optimisation level, the same does not apply to sample ablation chambers. High removal rate, high efficiency (i.e. complete transport of the ablated material) and reduced memory effects still represent problematic issues that calls for further development of the existing systems. Accordingly, several cell configurations with different geometries have been devised in the last decades, with the main goal being the optimisation of gas-flow patterns (see (1,2) as some representative examples).

Here we would like to present a new cell design developed in our labs which enables a homogeneous and fast removal (lower than 200 ms) from a sample cylindrical chamber with an internal diameter of 70 mm diameter. These results were achieved by combining the optimisation of the flow pattern inside the sample chamber,with an extraction tube coaxial with the laser beam and thus constantly positioned on the ablation spot. The cell sealing is warranted by a viscous film junction between the cell floor and cover. Optimisation and performances of the apparatus are discussed in detail together with possible future implementations of this design.

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NON-TARGET SCREENING OF THE PHOTODEGRADATION PRODUCTS FORMED IN A BEVERAGE CONTAINING ALLURA RED DYE

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Previous studies have shown that some food dyes present in commercial beverages undergo degradation for the action of sunlight (1). Aim of our study is to mimic the action of uncontrolled sunlight as it can be encountered during transport, distribution and storage of beverages, in order to identify the photodegradation products. In this work we consider a drink produced for children, that is declared to contain water, sucrose, citric acid, ascorbic acid, sodium chloride, strawberry juice, extract of chamomile flowers and Allura Red AC (E129) as colorant. It is worthwhile to underline here that Allura Red AC is reported as particularly dangerous for children, provoking “effects of hyperactivity and loss of attention” (2).

The study, performed by simulated sunlight photoirradiation, is carried out by UHPLC-MS/MS technique, comparing the performances of the low resolution hybrid triple quadrupole/ion trap mass analyzer 3200 QTrap^{TM} and the high resolution 5600 TripleTOF^{TM}. For the identification of the photodegradation products, the software tool Information Dependent Acquisition (IDA) was used both to automatically obtain information about the species present and to build a multiple reaction monitoring (MRM) method with the MS/MS fragmentation pattern of the species considered.

The use of TripleTOF^{TM} high resolution mass spectrometer helps in the elucidation of the unknown chemical structures also by using powerful software to mine the recorded chromatogram.

The results show that the identified degradation products derive from side-reactions and/or from interactions among the dye and other ingredients present in the beverage. The degradation pathway is therefore strictly dependent on the beverage composition.

As it concerns the effects on consumer health, the presence of aromatic amine or amide groups in the chemical structures of the degradation products suggests a potential hazard.

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RAPID EXTRACTION METHOD FOR GC/MS DETECTION OF ENVIRONMENTAL POLLUTANT RESIDUES IN HUMAN FETAL AND NEWBORN TISSUES.

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Sudden Infant Death Syndrome (SIDS) and Sudden Intrauterine Unexplained Death (SIUD) represent a relevant death-causing syndromes in developed countries. Due to a lack of detailed postmortem studies and not yet determined environmental co-factors, their etio-pathogenetic factors are still unknown (1,2). Literature data demonstrate the negative effect of the exposure to environmental pollutant residues (4,5) on fetal growth and brain development. However, information regarding the detection of these pollutants in SIUD and SIDS autopsy findings is still missing. The aim of this study is to develop a rapid extraction method for the determination of a notable number of environmental pollutant residues in human fetal tissues from subjects died *sine causa* after the 25th gestational week and SIDS victims. The extracts will be analyzed by GC-MS (6). The approach is based on a simple double ultrasonic bath extraction using a solvent mixture composed by hexane/dichloromethane (1:1,v/v), followed by a SPE clean up using Florisil and silica cartridge in serial connection. Nine isotopically labeled internal standards were added before extraction for quantitative purposes. The method was validated in terms of accuracy, precision, LOQ, LOD and linearity using fetal and newborn tissues (liver and brain) spiked at three concentration: 60 ng/g, 180 ng/g, 360 ng/g. This simple and rapid extraction gave good repeatability, reproducibility and high extraction efficiency in a wide range of concentrations. Results on environmental pollutant exposure can provide a framework to investigate possible environmentally induced alterations on fetal and neonatal development for a better understanding of the pathogenetic mechanisms leading to SIDS and SIUD.

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