Chimica Analitica

ANA-KN-01 Functionalized carbon nanostructures and metal nanoparticles: from effective charge propagation to enhancement of electrocatalytic, photoelectrocatalytic and bioelectrocatalytic properties

Pawel J. Kulesza

Department of Chemistry, University of Warsaw, Pasteura 1, PL-02-093 Warsaw, Poland
* *pkulesza@chem.uw.edu.pl*

Of particular interest to the preparation of advanced materials is synthesis and characterization of carbon nanostructures (e.g. nanotubes) and noble metal nanoparticles, their stabilization (e.g. through self-assembly), as well as organization into two-dimensional arrays and controlled fabrication (e.g. through the sequential attraction) into three-dimensional network films. They can form nanosized materials with well-defined composition, structure and thickness. The interfaces can be also highly functionalized, and they can exhibit specific catalytic or unique electronic, charge storage, optical and sensing properties. We explore here the ability of inorganic structures to stabilize and derivatize metal and carbon nanostructures. Among inorganic systems, polyoxometallates of molybdenum and tungsten are attractive since they can not only adsorb irreversibly on solid surfaces but also exhibit reversible stepwise multielectron transfer reactions. The concept of the layer-by-layer formation of hybrid (organic-inorganic) assemblies composed of anionic polyoxometallate-protected carbon nanotubes (or metal nanoparticles) and ultra-thin films of positively charged conducting polymers (e.g. such as polyaniline or PEDOT) will be described and discussed here. The resulting novel composite materials have been fabricated as thin or moderately thick (μm level) films on electrode surfaces. As evidenced from STM and scanning electron microscopy, their morphology is still granular but the structure is fairly dense. Further, they are characterized by fast dynamics of charge propagation. Obviously, this research is of importance to the construction of effectively operating charge storage devices (capacitors), charge mediators (e.g. in bioelectrochemistry), molecular electronic systems and electrocatalysis. In the latter case, polyoxometallates can also be applied to stabilize and link Pt-Ru, Pt-Sn and various alloyed Pt-based nanoparticles. It is apparent from diagnostic cyclic voltammetric, rotating disk voltammetric and chronoamperometric measurements that such systems exhibit attractive properties towards electroreduction of oxygen or oxidation of alcohols (ethanol, methanol). Here, it is possible that addition of polytungstate or molybdate clusters to ruthenium or tin hydroxo species at the catalytic interface results in activating effect on dispersed platinum particles. An alternate explanation may involve a possibility of electronic effects and/or different morphologies of the catalytic films in the presence and absence of polyoxometallate.

ANA-KN-02 Comprehensive Chromatography (GCxGC, LCxLC) coupled to Mass Spectrometry for the Analysis of Complex Matrices

Luigi Mondello
Dipartimento Farmaco-chimico, Università di Messina, Viale Annuziata – 98168 Messina, Italia

Comprehensive two-dimensional GC (GCxGC)[1,2], up to now the most used comprehensive chromatographic technique, presents the advantages of an enhanced resolving power, the chromatogram-formation of chemically-similar compound patterns, of great help in identifying "unknowns", and enhanced sensitivity through solute band re-concentration. Its main limitations are that retention mechanisms are more or less dependent on solute vapour pressures, and that only volatile, thermally-stable components can be analysed. These limitations can be overcome by using comprehensive two-dimensional LC (LCxLC) [3,4], a technique which is undergoing a very wide diffusion due to its great potential. The number of LC modes with distinct separation mechanisms is greater, and hence the number of possible orthogonal combinations is higher. The present contribution is focused on the use of comprehensive chromatographic methods applied to the analysis of complex mixtures with emphasis to the coupling of these techniques with Mass Spectrometry detection such as the one reported in Figure 1 for the Comprehesive LCxLC separation of intact triacylglycerols.

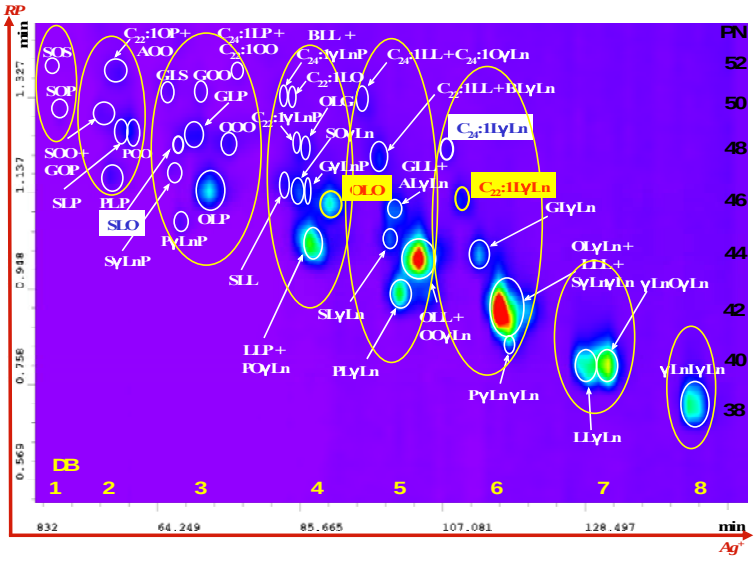


Figure 1

[1] Tranchida, P. Q.; Casilli, A.; Dugo, P.; Dugo, G.; Mondello, L. *Anal.Chem.* **2007,** *77*, 2266-2275.
[2] Mondello, L.; Tranchida, P. Q.; Dugo, P.; Dugo, G. *Mass Spectrom. Rev.* **2008,** *27*, 101-124.
[3] Dugo, P.; Favoino, O.; Luppino, R.; Dugo, G.; Mondello, L. *Anal. Chem.* **2004,** *76*, 2525-2530.
[4] Mondello, L.; Herrero, M.; Kumm, K.; Dugo, P.; Cortes, H.; Dugo, G. *Anal. Chem.* **2008**, *80*, 5418-5424.

ANA-OR-01 Detection Of Benzo[a]Pyrene Oxidation Products Via Electrochemical DNA Biosensors

V. Lanzone, M. Del Carlo, L. Ferretti, M. Sergi, D. Compagnone

Dipartimento di Scienze degli Alimenti, Universita´ degli studi di Teramo, Via Carlo LERICI, 1, 64023 Mosciano Sant'Angelo (TE), Italy

The toxic effect of polycyclic aromatic hydrocarbons (PAHs) occurs via their activation to more reactive species, usually oxidized species, via metabolic pathways. Benzo[a]pyrene is generally considered as one of the more toxic PAHs. In the present study we report the use of DNA biosensors based on screen printed electrodes for the indirect detection of the oxidized form of Benzo[a]pyrene. Benzo(a)pyrene-r-7,t-8-dihydrodiol-t-9,10-epoxide (DE-BAP) and Benzo[a]pyrene oxidized using a protocol standardized in our lab were the target molecules. Two different approaches have been tested: the use of genomic DNA sensors and the use of hybridization DNA sensors. In the former approach a different electrochemical signal was evidenced for benzo[a]pyrene vs. benzo[a]pyrene oxidized products.
The use of oligonucleotides consisted in the design of an electrochemical biosensor able to generate an electrochemical response for a particular sequence of DNA upon hybridisation. The same sequence, after formation of adducts with oxidized benzo(a)pyrene, was supposed to give a decreased signal with respect to the native.
Different DNA probes have been designed, immobilized via thiol-gold on gold screen-printed electrodes and gold nanoparticles. Amplification of the signal has been obtained using a biotinylated complementary probe in conjunction with a streptavidin-alkaline phosphatase conjugate.
An interesting correlation exists between DE-BAP concentration and the inhibition of the hybridization reaction for 24-mer oligonucleotides immobilised onto screen printed gold electrodes. Experiments on oligonucleotides immobilised on gold nanoparticles are in progress. The approach reported appears very promising for the realisation of DNA sensors able to assess the potential toxicity of PAHs and other genotoxic molecules.

ANA-OR-02 Proteins integrated into organic field effect transistor as electronic biosensors

D. Angione[1], N. Cioffi[1], S. Cotrone[1], M. Magliulo[1], A. Mallardi[2], L. Sabbatini[1], G. Palazzo[1]*, L. Torsi[1]*

[1] Dip. Chimica, Università di Bari, Via Orabona, 4, I-70126 Bari, Italy.
[2] Ist. per i Processi Chimico-Fisici, CNR, Via Orabona, 4, I-70126 Bari, Italy.

**corresponding authors e-mail: torsi@chimica.uniba.it; palazzo@chimica.uniba.it*

To satisfy the demand for smart analytical systems a great interest has been focused on the development of novel biosensors and several devices have been proposed. However, miniaturization, signal amplification and label-free detection are still open issues. Organic Field Effect Transistors (OFETs) appear as a new class of sensors able to overcome some of the presently available biosensors drawbacks. They have already demonstrated the ability to electronically detect numerous analytes in vapour systems [1] and even to detect chiral compounds at unprecedented low concentrations [2]. OFET sensors allows for simple and low-cost fabrication techniques, miniaturization, multi-parametric responses, signal and response amplification as well as label-free detection. Proteins such as antibodies, receptors or enzymes, can be exploited as highly performing recognition elements in OFET sensing devices. In this presentation an overview of the Bio-FET sensors (not necessarly organic based) field will be presented showing the advancements of the last years. Besides, the integration of biological recognition elements such as antibodies or other proteins to confer specificity will be discussed. Focus will be on the coupling of the FET device transduction mechanism and the biological recognition system. Recent achievements obtained with organic semiconductor FET biosensors realized through the full integration in the electronic device of the biological recognition elements will be also presented [3, 4]. The coupling of the OFET device and the biological recognition system is actuated by assembling supra-molecular structures in which biomolecules, such as membranes and proteins become an integral part of the OFET active material. Specific reactions (i.e receptor/analyte binding) are then used for analyte detection. To perform the bio-sensing measurements, the solution containing the analyte is deposited on the organic semiconductor, or through a proper microfluidic system. Preliminary results show that such bio-electronic devices can be very selective reaching detection limit (LOD) in the low ppt range. In addition, such sensors allow for label-free detection.

[1] L. Torsi and A. Dodabalapur, Anal. Chem. 70, 381A-387A (2005).
[2] L. Torsi, et al. Nat Mater., 7, 412 – 417 (2008).
[3] L. Torsi et al. European Patent. EP 10425146.7 – Submitted May 2010.
[4] M.D. Angione et al. submitted.

ANA-OR-03 Graphene-based modified electrodes for the determination of strong oxidants

F. Terzi, [a] C. Zanardi, [a] B. Zanfrognini, [a] J. Pelliciari, [a] R. Seeber, [a] A. Viinikanoja, [b] Jukka Lukkari, [b]

[a] Dipartimento di Chimica dell'Università di Modena e Reggio Emilia, Via Campi 183, 41125, Modena, Italy
[b] Department of Chemistry, University of Turku, FIN-20014, Turku, Finland
fabio.terzi@unimore.it

Graphene-based systems constitute a novel, potentially powerful family of materials. At present they are extensively investigated for a number of different applications, such as energy storage and conversion and catalysis. In addition they appear to be promising materials for the modification of electrode surfaces, aiming at the amperometric determination of a number of different species.

In the present contribution, a graphene-based material have been employed for the modification of Au electrodes. Soluble oxidised graphene have been prepared through graphite exfoliation. Then graphene oxide "aggregates" have been anchored on Au electrode by means of thiol molecules. Finally, the aggregates have been electrochemically reduced. Atomic force microscopy and surface-enhanced Raman spectroscopy have been employed to confirm the deposition of the aggregates while electrochemical tests have demonstrated the stability of the grafting.

The modified electrodes have been succefully employed for the determination of strong oxidising species in aqueous solution, such as hydrogen peroxide. The determination of these species is crucial in order to achieve a reliable process control in a large number of industrial sectors. Different detection systems have been developed so far, the spectrophotometric methods being most popular. At the moment, methods that are reliable, selective and simple at the same time are still absent. In this frame, the development of amperometric sensors for oxidising species represents a significant innovation: similar systems are easy to use, cheap and potentially portable. They can also be employed for online measurements, as well as detectors in chromatographic systems.

Au, Pt, Hg and carbon-based electrodes have shown to constitute interesting sensing probes for these species. However, these materials lack of selectivity, being unsuitable to distinguish among the different oxidising species present in the samples at the same time. In addition, the presence of interfering species, such as oxygen, prevents from application to real matrices.

In the case of hydrogen peroxide, graphene modified electrodes exhibit promising performance with respect to the conventional electrode materials previously reported. In particular, a significant shift of the reduction potential towards less negative values has been observed, indicating the activation of electrocatalytic charge transfer processes. Oxygen presence does not interfere with the hydrogen peroxide electrochemical response.

ANA-OR-04 Gold Nanostructures for Affinity Biosensing Applications

Andrea Ravalli[a], Glauco Pilon dos Santos[b], Hideko Yamanaka[b], Giovanna Marrazza[a]

[a] Dipartimento di Chimica dell' Università di Firenze, Via della Lastruccia 3, 50019, Sesto Fiorentino (FI), Italy
[b] Department of Analytical Chemistry, São Paulo State University (UNESP), Rua Prof. Francisco Degni, s/n – 14800-900 – Araraquara/SP, Brazil
andrea.ravalli@unifi.it

Recent advances on nanotechnology have led to discovery of many nanoscale materials (whose size is between 1 and 100 nm), because they present properties that are significantly different respect to bulk materials due to their dimensions (i.e.: large fraction of surface atoms, large surface energy, spatial confinement and reduced imperfections[1]).

In particular, gold-based nanomaterials (i.e.: gold nanoparticles, gold nanorods, etc.), that show unique optical, electronical, catalytical properties combined with easy functionalization with biomolecules and low toxicity, have been extensively used in biological field. In particular the combination of nanomaterials with bio-assays offer the possibility to design optimal sensing devices able to facilitate disease diagnosis (molecular diagnostics) as well as therapy optimisation (theranostic).

The goal of this work was to design a gold-based nanomaterial sandwich assay for the detection of different tumor markers (such as carbohydrate antigen CA 125, human epidermal growth factor HER2, prostate-specific antigen PSA etc), exploiting the easy functionalization of gold nanostructures with a specific antibody anti-marker.

Each phases of sandwich assay have been studied and optimized in order to increase the sensitivity and the reproducibility. For this purpose optical and electrochemical techniques were used.

[1] G. Cao, *Nanostructures & Nanomaterials: Synthesis, Properties & Applications,* Imperial College Press, **2004**

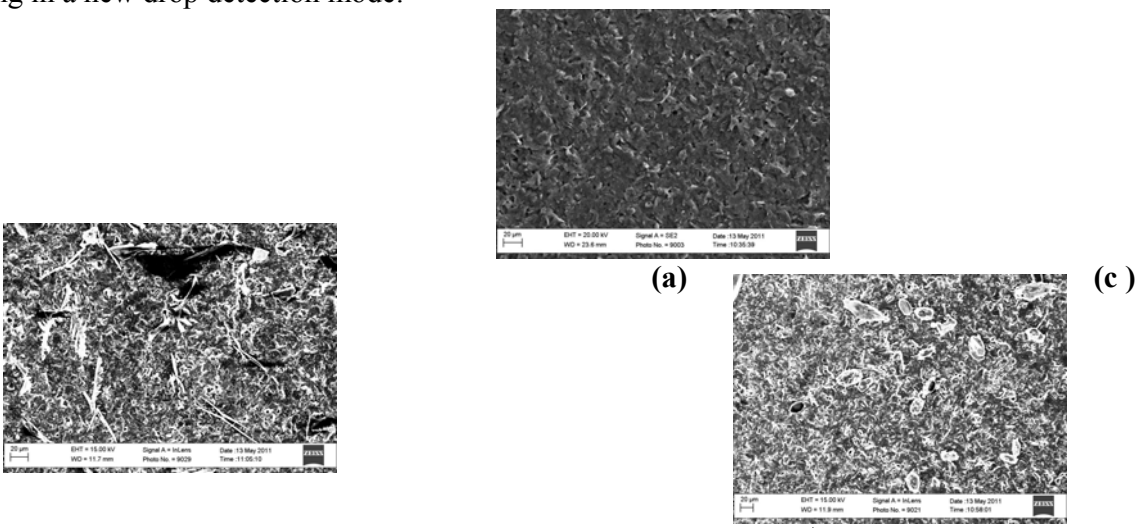
ANA-OR-05 Functionalization of Graphene Nanoribbons in Ionic Liquids: Myoglobine based biosensors and Prussian Blue modified chemical sensors.

Federica Valentini[1], Davide Roscioli[1], Marilena Carbone[1], Valeria Conte[1] , Barbara Floris[1] and Giuseppe Palleschi[1]

[1] Dipartimento di Scienze e Tecnologie Chimiche, Università Tor Vergata, via della Ricerca Scientifica 1, 00133 Rome

e-mail: federica.valentini@uniroma2.it

Following early attempts for producing graphene by mechanical exfoliation of highly oriented pyrolitic graphite (HOPG), many research groups are seeking high-throughput processing routes. In this paper, we propose an ILs-assisted electrochemical exfoliation of graphite electrode to obtain stable and homogeneous nano-emulsions of graphene nanoribbons and to perform electrochemical synthesis of graphene nanomaterials. Ionic liquids are salts containing organic cations, whose melting point is below 100 °C, most importantly, ILs have surface tensions very close to the surface energy of graphite, which is a solvent key prerequisite for direct exfoliation of graphite. In addition, the basic structural attribute of ILs (their ionicity), appears to be a unique feature for stabilization of exfoliated graphene via Coulombic interactions. Such advantages over most solvents make ILs the ideal systems for graphene synthesis. In the present study, a characterization of the new graphene based nanomaterials has been carried out under a topographic (by SEM/EDX) and structural (by FT-IR, UV-Visible and XPS) point of view. Subsequently, the [BMIM$^+$][Cl$^-$] (1-butyl-3-methylimidazolium chloride) and the [Bupy$^+$][Cl$^-$] (1-butylpyridinium chloride), were used to disperse the oxidized graphene nanoribbons, prepared as described in our previous paper [1], where the Myoglobine (Myb) protein has been successfully immobilized by physisorption interaction [2]. This biosensor, is very useful to detect NO_2^-,an environmental pollutant, and H_2O_2, an electroactive probe and substrate, with an improved Signal/Noise ratio, sensitivity and Limit of detection (L.O.D.), thanks to the extraordinary electronic properties of graphene nanoribbons and their higher surface area as compared with the conventional electrode materials. Moreover, the H_2O_2 electro-analytical detection could be improved, by using the one-step-electro-synthesis of the Prussian Blue (an electrochemical mediator-PB), directly electro-deposited on the SPE surface (Screen Printed Electrodes; ϕ=3mm) previously modified with the [Bupy$^+$][Cl$^-$] and BMIM$^+$[Cl$^-$] /graphene nanoribbon dispersions. Finally, the H_2O_2 and NO_2^- electrochemical measurements were performed working in a new drop detection mode.



(b) **(a)** **(c)**

Figure 1. SEM micrographs of (**a**): the bare SPEs, (**b**): the[Bupy$^+$][C]/graphene nanoribbons modified SPEs; and finally (**c**): the [BMIM$^+$][Cl$^-$]/graphene nanoribbons modified SPEs.

[1]. F. Valentini, G. Palleschi, et al.; *Carbon* 4 8 , **2 0 1 0**, 2 5 9 6 –2 6 0 2.

[2]. A. C. Dale Brownson, and C, E. Banks, Analyst 135, **2010**, 2768–2778.

ANA-OR-06 Rapid and efficient size exclusion sample treatment for the LC-MS/MS multi allergen detection in food by targeted proteomic

C. Bignardi[1], M. Mattarozzi, A. Penna, L. Elviri[1], M. Careri[1], A. Mangia[1]

[1] Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Università degli Studi di Parma, Parco Area delle Scienze 17/A, 43124, Parma, Italia.

Hidden allergens in foods represent a major health problem in the food safety issue. In particular, tree nuts are considered among the major food allergens and their presence in foods could be intentional or could occur accidentally *via* cross-contamination at any stage of food production. Legislation in several countries, especially in the USA and EU, was put in place to increase food safety [1]. Analytical methods for qualitative and quantitative determinations of food allergens including immunochemical and mass spectrometric-based methods have been recently reviewed by Kirsch et al. [2]. In this context, our research group successfully proposed analytical methods using liquid chromatography-electrospray ionization-tandem mass spectrometry by selecting univocal biomarker peptides of digested proteins for reliable allergen quantification in foods [3-5]. In this work, fast shot-gun selected reaction monitoring methods were developed for the reliable and efficient detection of Ana o 2 (cashewnut), Cor a 9 (hazelnut), Pru 1 (almond), Jug r 4 (walnut) and Ara h3/4 (peanut) allergens in complex matrices as dark chocolate, biscuits and cereals. A rapid size-exclusion solid phase extraction-based procedure was devised enabling allergen detection in the 0.1-1.3 mg nut/kg range for biscuits and 4-11 mg nut/kg range for dark chocolate. Precision in terms of intra-day repeatability and intermediate precision was always lower than 19% (RSD). Linearity was demonstrated up to about three orders of magnitude for each matrix. Assay recovery was in the 84(±6)-106(±4) % and 98(±5)-108(±6) % range for biscuits and dark chocolate, respectively. Finally, the method was successfully applied for the investigation of hidden nut traces in commercially available foodstuff of different brands aiming to ascertain possible discrepancies between food content and labels.

[1] Commission, E., *Off. J. Eur. Union,* **2006**, L 368:110.
[2] S. Kirsch, *Anal. Bioanal. Chem., 395,* **2009**, 57.
[3] M. Careri, *Anal. Bioanal. Chem., 38,* **2007,** 1901.
[4] M. Careri, J. *Chromatogr. A,* 1206, **2008**, 89.
[5] C. Bignardi, *J. Chromatogr. A, 1217,* **2010,** 7579.

ANA-OR-07 Differential label free quantitative analysis of protein corona adsorbed onto different non-viral gene delivery systems

Chiara Cavaliere,[a] Anna Laura Capriotti,[a] Giulio Caracciolo,[b] Daniela Pozzi,[b] Roberto Samperi,[a] Aldo Laganà[a]

[a] Dipartimento di Chimica della Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185, Rome, Italy
[b] Dipartimento di Medicina Molecolare della Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185, Rome, Italy
chiara.cavaliere@uniroma1.it

One of the most important requirements for gene therapy is the development of safe and efficient gene delivery systems. In the last two decades non-viral vectors have attracted a growing interest. The cationic liposomes (CLs) have been extensively studied as non-viral vectors since the first lipofection was reported in 1987 [1]. One of the most common non-viral gene delivery vectors is DNA-cationic lipid complex (lipoplex). Medical administration of these gene delivery vectors is frequently made by parenteral injection. Therefore, upon exposure to biological media, these are immediately covered by plasma proteins forming a rich "protein corona" [2]. Indeed, the binding of plasma proteins to nanoparticles, is a critical step in determining their fate in vivo.

A shotgun proteomics approach was used to characterize and compare the plasma proteins constituting the protein corona adsorbed onto CLs, CL-DNA complexes (lipoplexes) and lipid/polycation/DNA (LPD) complexes surface after contact with plasma. The nanoparticle-protein complex was separated from plasma by centrifugation, then the proteins were digested, and the resulting tryptic peptides were analyzed by nano-high performance liquid chromatography coupled to a high resolution Orbitrap mass spectrometer. This is the first study that characterizes the "protein corona" of CLs, lipoplexes and LPD complexes with this approach. We found that these nanoparticles bind different plasma protein categories with important biological functions, e.g. lipoproteins, immunoglobulins, acute-phase proteins, proteins playing an essential role in protein synthesis, proteins strongly related to cellular activity and proteins involved in complement pathways and coagulation. After identification, the proteins present in at least two different nanoparticle corona were quantified by means of the statistical software Sieve.

These results could help in designing gene delivery systems able to bind selectively certain proteins rather than others, and to drive their biodistribution *in vivo* for obtaining more efficient and effective gene therapy.

[1] P.L. Felgner, T.R. Gadek, M. Holm, R. Roman, H.W. Chan, M. Wenz, J.P. Northrop, G.M. Ringold, and M. Danielsen, *Proc. Natl. Acad. Sci. U.S.A.*, *84*, **1987**, 7413.
[2] I. Lynch, and K.A. Dawson, *NanoToday, 3*, **2008**, 40.

ANA-OR-08 Synthesis and Analytical Characterization of Composite Nanomaterials for NO_x Sensors

A. Afzal[a], N. Cioffi[a,b], C. Di Franco[c], N. Ditaranto[a], E. Mesto[d], F. Scordari[d], G. Scamarcio[c,e], L. Torsi[a,b]

[a] Department of Chemistry, University of Bari, via Orabona 4, 70126 Bari, Italy
[b] TIRES Center of Excellence, University of Bari, via Orabona 4, 70126 Bari, Italy
[c] CNR-INFM Regional Laboratory LIT3, via Amendola 173, 70126 Bari, Italy
[d] Department of Heart and Geoenvironmental Sciences, University of Bari, via Orabona 4, 70126 Bari, Italy
[e] Department of Physics, University of Bari, via Amendola 173, 70126 Bari, Italy
cioffi@chimica.uniba.it

The control of pollutants emission from internal combustion engines is a worldwide issue, in the automotive field. The roadmap for the reduction of vehicle emission limits is driving the academic and industrial interest towards the development of innovative systems integrating novel detection elements and fast feedback circuits and actuators. Based on a tighter control over emissions, and starting from 2014, Euro 6 standards are expected to improve the environmental compatibility of a new generation of vehicles in Europe. This scenario calls for a significant improvement of the sensors technologies for the detection of the main pollutants related to the automotive field, including nitrogen oxides (NO_x).
In this work, we report on the synthesis and analytical characterization of hybrid nanocomposites containing gold nanoparticles (Au-NPs) and metal oxide nanostructures (MO-NPs, such as zirconium oxide, indium oxide, oxide mixtures, etc.). These species are promising for real-time detection of low levels of NO_x species, owing to their low cost, high sensitivity and availability under a variety of stoichiometric and mixing ratios, showing different gas sensing characteristics [1-2]. Different MO-NPs and mixed MO-NP systems were prepared using a simple but efficient sol-gel method. Subsequently, the nano-oxides were electrodecorated by Au-NPs. Since Au nanophases exhibit pronounced selectivity toward NO_x gases [3], the resulting hybrid nanocomposites are expected to improve the nanomaterial sensing performance. All the nanomaterials were characterized using FTIR, XPS, XRD, TEM, and SEM techniques. Experimental evidences support further application of these NPs as active elements in novel NO_x sensors.

[1] K. J. Choi, H. W. Jang, *Sensors 10*, **2010** 4083.
[2] C. Di Franco, A. Elia, V. Spagnolo et al., *Sensors 9,* **2009** 3337.
[3] "Advanced NOx Sensors for Mechatronic Applications", A. Elia, C. Di Franco, A. Afzal, N. Cioffi, L. Torsi, in "Mechatronics", ISBN: 978-953-307-373-6, InTech Publisher, **2011**, in press

ANA-OR-09 Surface Plasmon Resonance imaging: improving analytical performances for DNA sensing applications

M.L. Ermini[a], S. Mariani, S. Scarano[a], M. Bonini[b] and M. Minunni[a,]

[a]Dipartimento di Chimica "Ugo Schiff", Università degli Studi di Firenze, Via della Lastruccia 13, 50019, Sesto F.no (FI), Italy
[b]CSGI, Università degli Studi di Firenze, Via della Lastruccia 13, 50019, Sesto F.no (FI), Italy
marialaura.ermini@unifi.it

In Surface Plasmon Resonance imaging (SPRi) based sensing, NPs can act as signal enhancers [1,2,3] or they can be used for nanostructured surfaces. An improvement in sensitivity is reported when nanoparticles (NPs) are exploited for functionalizing the interacting surface [1,2,4] using NPs on gold electrodes coupled to piezoelectric sensing (QCM) [3]. Sharpening the performances of a sensor is a prominent objective in developing innovative biosensors for clinical applications. SPRi technique proved to be a suitable asset for developing versatile DNA affinity biosensors. Probe-target interactions can be monitored in real time and simultaneously recorded as a sensorgram (intensity of reflected light % vs time) or real time image of the interacting surface. A differential image of the functionalized biochip renders the changes on the interacting surface in a color scale monitoring interactions in real-time.

The possibility of multi-analyte detection using an array format is very attractive [4]. In particular, current research is focused on very sensitive molecule detection i.e. target sequences analysis directly in genomic DNA.[2]
With the final aim to improve analytical performances of SPRi-based sensing, a nanostructure-modified surface's was developed and the sensing behavior studied. As model system polymorphism detection in the gene of the opioid receptor was applied. The detection of this polymorphism is used for theranostic approaches, i.e. for a tailored pain treatment.

Different probes, selected computationally, were immobilized on the surface via thiol chemistry and hybridization reaction recorded. The system analytical performances are evaluated.

[1] J. Jung, K. Na, J. Lee, Ki-Woo Kim, J. Hyun, Analytica Chimica Acta, 651, **2009,** 91–97.
[2] R. D'Agata, R. Corradini, C. Ferretti, L. Zanoli, M. Gatti, R. Marchelli, G. Spoto, Biosensors and Bioelectronics, 25, **2010,** 2095–2100.
[3] T. Liu, J. Tang, L. Jiang, Biochemical and Biophysical Research Communications, 313, **2004,** 3–7.
[4] F. Pillet, C. Thibault, S. Bellon, E. Maillart, E. Trévisiol, C. Vieu, J. M. Francois, V. A. Leberre, Sensors and Actuators B, vol. 147, **2010**, pp. 87–92.

ANA-OR-10 Development and characterization of electrosynthesized polypyrrole on microstructured silicon prepared by electrochemical micromachining

E. Mazzotta,[a] C. Malitesta,[a] S. Surdo,[b] L.M. Strambini,[b] G. Barillaro[b]

[a] Dipartimento di Scienza dei Materiali, Università del Salento, via Monteroni 73100 Lecce, Italy
[b] Dipartimento di Ingegneria dell'Informazione: Elettronica, Informatica, Telecomunicazioni, Università di Pisa, via G. Caruso 16 56126 Pisa, Italy
elisabetta.mazzotta@unisalento.it

Polypyrrole (PPY) is an electroactive conducting polymer widely used in various research and commercial fields from coating technology to sensor applications [1-4]. In particular, the electrochemical synthesis represents an effective way of easily interfacing PPY with an electrode surface allowing the deposition of films with controlled thickness even on complex geometry surfaces [5]. Recently, research has been devoted to the miniaturization of electrosynthesized PPY-based devices [6, 7] with the aim to construct complex micrometer- and nanometer-scale systems offering advantages in view of their larger surface area and, in turn, of the higher rate of interface processes. A polymeric thin film having both high conductivity and fine structure at the micro- or nanoscale is a suitable material particularly as sensing element in the fabrication of sensor devices [6, 7]. Various methods for preparing micro- and nanostructured PPY films have been proposed, which are generally based on template-assisted synthesis [7] exploiting carbon nanotubes [8], porous alumina templates [9], and porous silicon [10]. A drawback of this method is that dimension and morphology of the PPY structures is limited by the template architecture. Moreover, in some cases, PPY electrosynthesis requires a preliminary modification of the template surface to make it conductive [9]. Finally, each polymerization step needs a single-use template that is removed after film deposition, typically by chemical etching.

The present work describes a novel approach for the development of microstructured PPY films. The proposed approach is based on PPY electrosynthesis on microstructured silicon substrates prepared by electrochemical micromachining [11,12]. Electrochemical micromachining is a low-cost high-flexible technique allowing for silicon microstructuring at the microscale [13]. The great flexibility of silicon micromachining techniques for the fabrication of three-dimensional microstructured systems is here conjugated, for the first time, with conducting polymers technologies, thus leading to the development of novel PPY films with three-dimensional features that can be selected on the basis of specific applications. Experimental conditions for PPY electrosynthesis on silicon substrates have been firstly selected and different thickness films have been prepared. The influence of silicon microstructure has been tested by performing

PPY electrosynthesis, under the same experimental conditions, on flat substrates and on silicon substrates integrating regular array of square-like pores with pitch of 8 μm, size (s) of 5 μm and depth (d) of 5 μm, 10 μm and 50 μm. Interestingly, Scanning Electron Microscopy (SEM) analysis revealed that a three-dimensional polymer structure perfectly replicating the silicon microstructure is achieved on micromachined substrates. An isotropic PPY growth, i.e. same growth rate both in the horizontal and vertical direction, occurs independently from the aspect-ratio (HR = d/s) of the microstructure (HR from 1 to 10). The resulting PPY layer uniformly covers the microstructured silicon surface with constant thickness. Evaluation of the film thickness by SEM analysis also allowed the correlation with the circulated charge during PPY electrosynthesis to be established. Chemical analysis on microstructured PPY films has been also performed by X-Ray Photoelectron Spectroscopy (XPS). Preliminary tests aiming to verify the sensing properties of the developed microstructured systems will be presented.

[1] A. Ramanavicius, A. Ramanaviciene, A. Malinauskas, *Electrochim. Acta, 51,* **2006**, 6025.
[2] T.W. Lewis, S.E. Moulton, G.M. Spinks, G.G. Wallace, *Synthetic Met., 85,* **1997**, 1419.
[3] H. Nguyen Thi Le, B. Garcia, C. Deslouis, Q. Le Xuan, *Electrochim. Acta, 46,* **2001**, 4259.
[4] S. Radhakrishnan, P. Somani, *Mater. Lett., 37,* **1998**, 192.
[5] C. Malitesta, I. Losito, P.G. Zambonin, *Anal. Chem., 71,* **1999**, 1366.
[6] Y. Chen, Y. Luo, *Adv. Mater., 21,* **2009**, 2040.
[7] Rajesh, T. Ahuja, D. Kumar, *Sensor Actuat. B-Chem., 136,* **2009**, 275.
[8] J.Wang, J. Dai, T. Yarlagadda, *Langmuir, 21,* **2005**, 9.
[9] H. Yan, L. Zhang, J. Shen, Z. Chen, G. Shi, B. Zhang, *Nanotechnology, 17,* **2006**, 3446.
[10] K. Fukami, F.A. Harraz, T. Yamauchi, T. Sakka, Y.H. Ogata, *Electrochem. Commun., 10*, **2008**, 56.
[11] G. Barillaro, A. Nannini, M. Piotto, *Sens. Actuat. B-Phys., 102,* **2002**, 195.
[12] G. Barillaro, A. Nannini, F. Pieri, *J. Electrochem. Soc., 149,* **2002**, C180.
[13] G. Barillaro, L.M. Strambini, *Electrochem. Commun., 12,* **2010**, 1314.

ANA-OR-11 Peptide Modified Gold Nanoparticles E-Nose For Food Analysis

D. Compagnone[a], G.C. Fusella[a], M. Del Carlo[a], P. Pittia[a] , C. Di Natale[b], L.Tortora[c], R. Paolesse[c]

[a]Dipartimento di Scienze degli Alimenti, Universita´ degli studi di Teramo, Via Carlo LERICI, 1, 64023 64023 Mosciano Sant'Angelo (TE), Italy
[b]Dipartimento di Ingegneria Elettronica ,Universita´degli Studi di Roma Tor Vergata, via del Politecnico, 00133 Roma, Italy
[c]Dipartimento di Scienze e Tecnologie Chimiche, Università degli Studi di Roma Tor Vergata, via della Ricerca Scientifica, 00133, Roma, Italy.
dcompagnone@unite.it

During food production the addition of aromatic compounds or the release of them during the technological steps has to be monitored for the assessment of the quality of the final product. The final aroma is the result of the entire process [1].
The complexity of the aromatic patterns released by food it is also dependent of the composition of the matrix and can be monitored using "electronic noses" [2].
In this study we added new functionalities to piezoelectric sensors by modification with gold nanoparticles (GNPs) bearing short peptide moieties.
GNPs has been synthesized using the $NaBH_4$ method that yields GNPs in alkaline solution. GNP are unstable due to their high surface energy and need to be stabilized against aggregation by suitable surface modifications. Some functional groups such as cyano (-CN), mercapto (-SH) and amino ($-NH_2$) are known to have an high affinity for gold [3]. The addition in homogeneous solution of compounds as cystein (CYS), glutathione (GSH), γ-glutammylcystein (γ-GLU-CYS) and cysteinylglycine (CYS-GLY) resulted in the formations of modified GNPs. Closely related aminocids were selected to assess potential relationships between structure and sensor behavior. The synthesized GNPs have been characterized using TEM, VIS spectroscopy and electrochemistry.
Modified sensors have been obtained by casting GNPs on 20 MHz quartz crystal microbalances.
The modified piezoelectric sensors have been characterized in the e-nose with different kinds of solvent to understand the interaction ability of each kind of sensors. Acqueous model solutions of aromas as isopentyl acetate, cis-3-hexen-1-ol, terpinen-4-ol, 2,3-pentandione, 2,3-butandione, hexanal and etylpyrazine were tested.
Headspace analysis of different samples of extravirgin olive oils were also tested and the data compared with GC and sensory analysis.
The data demonstrates that this approach is useful to improve the performance of QCM based e-noses. Particularly, for acqueous solutions the discrimination ability obtained by a simple Principal Component Analysis appears similar to the well known porphyrin–based QCMs for acqueous samples. Improved ability to

discriminate vs. porphyrin based e-nose was observed for extra-virgin olive oil samples; in this case the peptide based e-nose was able to clearly distinguish defected samples.

REFERENCES

[1] B. Plutowska, W. Wardencki, Food Chemistry, 101, **2007**, 845;

[2] M. Santonico, P. Pittia, G. Pennazza, E. Martinelli, M. Barnabei, R. Paoloesse, A. D'Amico, D. Compagnone, C. Di Natale, Sensors and Actuators B, 133, **2008**, 345;

Santosh, B.K.C Remant, N. Dharmaraja, B. Narayan, K. Chi Hui, K. Hak Yong, Spectrochimica ACTA part A, 63, **2006**, 160.

ANA-OR-12 Rationalization of the behaviour of a bi-label oxygen optical sensor

Denis Badocco, Andrea Mondin and Paolo Pastore
Department of Chemical Sciences, University of Padua, Via Marzolo 1, 35131 Padua, Italy
andrea.mondin@unipd.it

The determination of oxygen concentration is important in many areas of industry, medicine and the environment. Oxygen optical sensors are more and more attractive than conventional amperometric devices, because, in general, they have a faster response time, they do not consume oxygen and are less easily poisonable. Sensor operation is based on the quenching of luminescence in the presence of oxygen. We rationalized the behaviour of an oxygen optical sensor made of two luminophores simultaneously embedded in polymeric matrices[1]. Theoretical findings were confirmed by experiments. Platinum(II) 5,10,15,20-tetraphenyl-21H,23H-porphyrin (PtTPP), Palladium(II) 5,10,15,20-tetrakis(pentafluorophenyl)-21H,23H-porphyrin (PdFTPP), Ruthenium (II) (4,7-diphenyl-l,l0-phenanthroline)$_3$ (octylsulfate)$_2$ (Ru(dpp)OS) were used as luminophores and embedded either in polyvinylchloride or in polysulfone matrices. Their different life-times allowed preparing sensing membranes having optimized precision in the required concentration interval by proportioning the luminophores relative amounts. We demonstrated that, in the experimental conditions adopted, the two luminophores behave as if they were independent, giving to the sensing layer enlarged working range with respect to the most sensitive membrane and improved precision with respect to the less sensitive membrane, as shown in Figure 1. A working curve may indicate the most suitable membrane composition. The choice of a bi-label sensor may be justified when it is necessary to detect oxygen in a chosen concentration interval with the best precision possible.

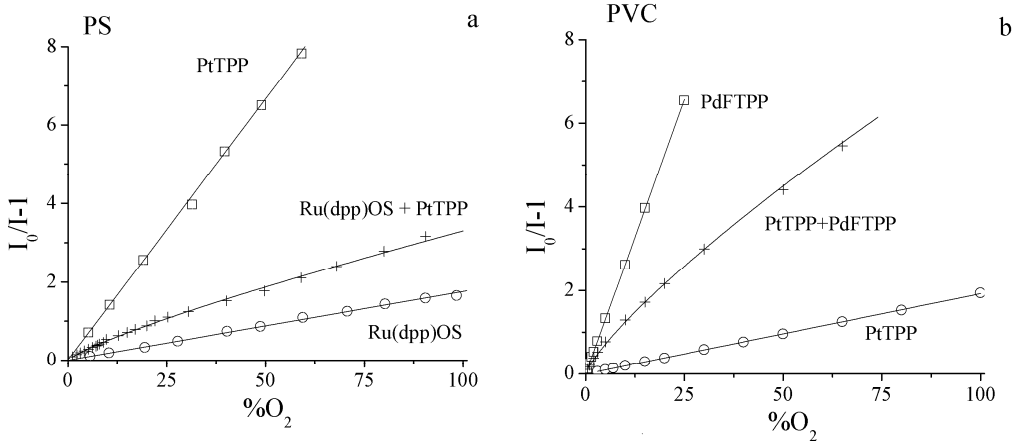


Figure 1.Stern-Volmer calibrations for single and bi-label systems in PS (a) and PVC (b) matrices

[1] D.Badocco, A.Mondin, P.Pastore Rationalization of the behaviour of a bi-label oxygen optical sensor, Sens. Actuators B: Chem. (2011), doi:10.1016/j.snb.2011.05.024

ANA-OR-13 Identification and Localization of Proteins in Painting Cross-Sections by Chemiluminescent Immunochemical Microscope Imaging

Massimo Guardigli,[a] Luisa Stella Dolci,[a] Giorgia Sciutto,[b] Angela Buragina,[a] Martina Zangheri,[a] Silvia Prati,[b] Rocco Mazzeo,[b] Aldo Roda[a]

[a]Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126, Bologna, Italy
[b]Microchemistry and Microscopy Art Diagnostic Laboratory (M2ADL), University of Bologna - Ravenna Campus, Via Guaccimanni 42, 48100, Ravenna, Italy
Massimo.guardigli@unibo.it

The characterization of the complex, multilayer, and multimaterial structure of a painting is fundamental for studying painting techniques and for authentication purposes. Among the various paintings' materials, organic ones (e.g., proteins, oils, waxes, gums, and resins) are the most difficult to characterize and their identification, localization and mapping in a painting structure still represents an analytical challenge. Imaging techniques relying on immunological reactions represent a promising approach to protein localization thanks to the high avidity and specificity of antibodies, which allow sensitive detection and (unlike other techniques such as Micro Fourier-Transform Infrared Spectroscopy) discrimination between different proteins. We have developed chemiluminescent (CL) immunochemical microscope imaging techniques for the identification and localization of ovalbumin and casein, commonly present in binding media or varnishes [1]. The immunological detection was performed by means of specific primary antibodies revealed by enzyme-labelled secondary antibodies and suitable enzyme CL substrates. The combination of CL imaging detection with optical microscopy permitted to localize the target proteins in micro cross-sections (1-2 mm^2) of standard and real aged painting samples with high sensitivity, low signal background and spatial resolution of the order of micrometers (i.e., within the single painting layers). Localization of bovine collagen has been also performed and multiplexed assays for the simultaneous detection of two different proteins have been developed by employing secondary antibodies labelled with enzymes detectable with different CL substrates. In perspective, these protein binders could be detected in the same cross-section by a triple multiplexed CL assay.

[1] G.Sciutto, L.S.Dolci, A.Buragina, S.Prati, M.Guardigli, R.Mazzeo, A.Roda, *Anal.Bioanal.Chem., 399*, **2011**, 2889.

Part of this research has been funded by the Italian Ministry of Instruction, University and Research (PRIN 2008 project, prot. 2008ZRSHHB) and by the European project "CHARISMA" FP7 INFRASTRUCTURE n.228330.

ANA-OR-14 Identification of viable pathogenic bacteria by an olfactory mos-based sensor array coupled with field-flow fractionation

Carolina Colliva[1], Barbara Roda[2], Pierluigi Reschiglian[2], Mara Mirasoli, Aldo Roda[1]

[1]*Department of Pharmaceutical Sciences, Via Belmeloro, 6, I-40126 Bologna, Italy, carolina.colliva2@unibo.it*
[2]*Department of Chemistry "G. Ciamician", Via Selmi, 2, I-40126 Bologna, Italy*
barbara.roda@unibo.it

Every year, almost 80 million cases of food borne illness occur in United States and 1.5 millions in Italy, among these 30% are caused by bacteria and their connected toxic products [1].
Conventional microbiological methods for the identification of food borne pathogenic bacteria are labor-intensive and time-consuming. Recently, immuno- or nucleic acid-based bioassay allowed significant reduction in the analysis time [2]. Nevertheless, their response does not provide information about viability and even dead bacteria are recognized. An approach based on bacteria metabolomics, which greatly differ among species, could be more reliable. Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) methods have been described, but due to high cost of instrumentation are not suitable for screening purposes [3]. The metabolomic approach can also be pursued employing electronic olfactory system (EOS), which can detect and classify volatile components, thus enabling rapid bacteria detection. However, EOS are not selective enough to identify bacteria when present in a complex mixture and a preliminary separation is required. Field-flow fractionation (FFF) are separative techniques suitable for the non invasive fractionation of bacteria. This work presents the use of an FFF system coupled with an EOS (EOS 835, SACMI, Imola, Italy) equipped with six metal oxide semiconductor sensors (MOS) for the analysis of volatile metabolites produced by pathogenic bacteria. The sensor technology yields a distinct response signature for each vapour regardless of its complexity, resulting in a "smell fingerprint" which can be used for sample identification by chemometric data analysis. To set up the method, *E. coli* O157:H7 and *Yersinia enterocolitica* were used as model samples. Upon training the EOS with suspensions of each bacteria species (2,4 X 10^9CFU/mL), cells mixtures with different bacteria proportions (1:4; 1:1; 4:1) were injected in an FFF system (final concentration 4,8 X 10^9CFU/mL). Fractions corresponding to the retention time typical for the two bacteria species were collected, grown in 1 mL Luria Bertani (LB) broth for 2h at 37°C, and then analyzed with the olfactory system. The data recorded for each sample were averaged and subjected to chemometric analysis using PARVUS software [4]. The selection of 10 variables allowed clearly discriminating by PCA the *Y.*

enterocolitica and *E. coli* samples when present in different relative proportion, and the LDA analysis allowed obtaining a correct classification and prediction ability of respectively 90 and 91 %. The analysis of collected fractions from the different mixtures confirmed that after fractionation, the olfactory system was able to distinguish and identify the different fractions. The inter-assay variability is low but a fully highly standardized procedure is required and this is achieved also with the use off the FFF system. This method could be applied for food safety applications, as well as to biological samples for diagnostic purposes and an on-line FFF coupling with EOS is in progress.

[1] PS. Mead, L. Slutsker, V. Dietz, LF. McCaig, JS. Bresee, C. Shapiro, *Emerg Infect Dis* 5, (1999) 607.
[2] M.Magliulo, P.Simoni, M.Guardigli, E.Michelini, M.Luciani, R.Lelli, A.Roda, J *Agric Food Chem*, 2007, 55(13): 4933-9.
[3] D.Parisi, M.Magliulo, P.Nanni, M.Casale, M.Forina, A.Roda, *Anal Bioanal Chem*, 2008, 391(6):2127-34.
[4] M. Forina, S. Lanteri, C. Armanino, M.C.C. Oliveros, C. Casolino. V-PARVUS 2004. Freely available at http://www.parvus.unige.it

ANA-OR-15 Study of the Vitamin K Cyclic Metabolism in absence and presence of coumarin anticoagulants by Liquid Chromatography- Linear Ion Trap Mass Spectrometry.

Alessandra Gentili[a]*, Giuseppe D'Ascenzo[a], Arturo Cafolla[b], Claudia Vigilante[a], Daniela Perret[a].

[a] Dipartimento di Chimica, Università "La Sapienza", Piazzale Aldo Moro 5, 00185 Roma, Italy.

[b] Dipartimento di Biotecnologie Cellulari ed Ematologia, Università "La Sapienza", Piazzale Aldo Moro 5, 00185 Roma, Italy.

e-mail: alessandra.gentili@uniroma1.it

In mammalians the only known function of vitamin K is that of γ-glutamylcarboxylase cofactor [1,2], enzyme catalyzing the posttranslational carboxylation of the so-called vitamin K-depending proteins [3]. Well-known for the activation of the coagulation proteins, vitamin K has also been shown to be required by extra-hepatic proteins involved in bone metabolism, vascular calcification, and apoptosis. This vitamin is characterized by a cyclic metabolism: it is its stable quinonic form to be absorbed and transported in blood, but it is the hydroquinone one to act as enzymatic cofactor and to be transformed into vitamin K 2,3-epoxide. The latter is then recycled to quinone and hydroquinone in successive reactions catalyzed by vitamin K reductases, dithiol-depending enzymes inhibited by coumarin drugs. A second NAD(P)H-dependent quinone reductase is relatively insensitive to these anticoagulants and operates at high concentrations of vitamin K.

Probably because of its efficient recycle system, very low levels of vitamin K circulate in plasma. Moreover, its Recommended Daily Allowance has currently been set at 1 μg/kg/day [1]; this value is surely suitable for its hepatic function, but an extra demand might be required for guaranteeing the bone and vessel health, especially in subjects under anticoagulant therapy [4]. For these reasons, an accurate determination of the vitamin K and its metabolites is a real analytical challenge. This work was just addressed to overcome the above-mentioned difficulties making use of an advanced analytical technique such as liquid chromatography-linear ion trap mass spectrometry. After its development and validation, the method was applied for refining the status of phylloquinone (vitamin K1) and for defining that of vitamin K1 2,3-epoxide in a significant cohort of healthy subjects and of patients under anticoagulant therapy. An accumulation of both forms was verified in individuals taking long-term coumarin drugs.

[1] M.J. Shearer, A. Bach, M. Kohlmeier, *J. Nutr*., **1996**, 126, 1181S.
[2] M.J. Shearer, P. Newman, *Thromb. Haemost*., **2008**, 100, 530.
[3] G. Ferland, *Nutr. Rev*., **1998**, 56, 223.
[4] E.C.M. Cranenburg, L.J. Schurgers, C. Vermeer, *Thromb. Haemost.***2007**, 98, 120.

ANA-OR-16 Determination of some phytohormones in Zea Mays under chemical and physical stress.

Ginepro Marco, Alessia Baima Peit, Marongiu Manuela, Zelano Vincenzo

Dipartimento di Chimica Analitica dell'Università degli studi di Torino
Via Piero Giuria n° 5, 10125, Torino, Italy
marco.ginepro@unito.it

As reaction to stress condition, plants can modify the amount of produced hormones. In this work we have considered the alteration of main phytohormones concentration in corn plants (Zea Mays) exposed to different types of stress. Some plants were exposed to water stress, other were fed with herbicide solutions (Flufenacet or Metolachlor) commonly used in corn cultivation.

The investigated hormones were *Indole-3-acetic acid* (IAA), *abscisic acid* (ABA) and *gibberellic acid* (GA3). The study of these hormones is particularly important because of their great physiological involvement in plants.

Hormones determination was done by HPLC-UV/ fluorescence and GC-MS; two different internal standards were used: ascorbic acid and naproxene. For HPLC-UV/fluorescence separation, a gradient procedure was adopted using acidified water (pH= 3) with phosphoric acid and acetonitrile. In fluorescence analysis, only IAA can be observed because it is the only hormone with fluorescence properties; as naproxene shows similar fluorescence properties, it has been chosen as internal standard. GC-MS analysis needs a preliminary stady about compounds derivatization with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) or with Trimethylsilyldiazomethane (TMSHN2).

Hormones extraction procedure from a so complex matrix was done with an aqueous solution of methanol 80% followed by a clean up on SPE column.

Compared with plants grown without stress, plant exposed to water stress shows an increase of ten times in IAA concentration; opposite trend is shown by ABA whose concentration decreases considerably. The same type of plant has a quite different behaviour when exposed to chemical stress. Plants fed with Metolachlor or Flufenacet show, in fact, a decrease of all hormones' concentration.

These results demonstrate that the same plant can react in different way depending on the different type of stress to which they are subjected.

ANA-OR-17 Correlation between salivary concentration of oral anticoagulants and anticoagulant effect in thrombotic patients.

Silvia Ghimenti[a], Massimo Onor[b], Tommaso Lomonaco[a], Roger Fuoco[a], Aldo Paolicchi[c], Lucia Ruocco[d], Giovanni Pellegrini[d], Maria Giovanna Trivella[e], Fabio Di Francesco[a].

[a] Department of Chemistry and Industrial Chemistry-University of Pisa, e-mail: *fuoco@dcci.unipi.it*
[b] Institute of Chemistry of Organometallic Compounds – CNR, Pisa
[c] Department of Experimental Pathology BMIE, sect. General and Clinical Pathology – University of Pisa
[d] Chemical-Clinical Analysis Laboratory – AOUP, Pisa
[e] Institute of Clinical Physiology – CNR, Pisa

Oral anticoagulants are essential and not replaceable in a large number of long-lasting clinical conditions which require an accurate control of the coagulation of blood, such as thrombotic diseases and vascular pathologies. Oral anticoagulants have such a narrow therapeutic index that small changes in plasma concentration may have serious consequences: bleeding if the dose is a little too high, or thrombosis if it is a little too low.
The large number of factors that may interact with the therapy (diet, comorbidities, other drugs, etc.) significantly increases the risk of being outside the optimal value, and consequently entails constantly monitoring the patient by means of continuous and frequent blood analysis, even for long periods of time. The dose is adjusted from time to time according to the anticoagulant effect, as evaluated by measuring in a blood sample the prothrombin time expressed as International Normalized Ratio (INR).
Clearly new alternative methods to blood tests are needed that would be less invasive, simple to use, implementable in low cost devices, and, if possible, allowing self-monitoring.
In this work, an analytical method is described for the determination of an oral anticoagulant (warfarin) in oral fluid samples by HPLC with a spectrofluorimetric detector. The correlation between the salivary warfarin concentration and INR values highlighted the key role played by the salivary pH.

ANA-OR-18 A multi-enzyme biosensor for detection of cyanobacterial hepatotoxins based on PP2A inhibition

G. Volpe, G. Di Pofi, D. Moscone, G. Palleschi

Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma Tor Vergata, Via della Ricerca Scientifica 1, 00133 Roma, giulia.volpe@uniroma2.it

Microcystins and nodularin are natural toxins produced by cyanobacteria such as *Microcystis*, *Oscillatoria*, *Anabaena* and *Nodularia*, which grow worldwide in fresh and brackish waters. They are potent hepatotoxic cyclic peptides that exert the cytotoxic effects by inhibiting the catalytic subunits of serine/threonine protein phosphatase-1 and 2A (PP1 and PP2A), which play essential roles in the reversible phosphorylation and dephosphorylation of proteins and are implicated in a large number of cellular events. Cyanobacteria and their toxins, especially microcystins, are recently a drinking water public health issue with a provisional drinking water guideline of 1 μg/L for microcystin-LR, published by World Health Organization. Rapid and reliable analytical methods capable of determining microcystins in water at concentrations ≤ of 1 μg/L are therefore required. A promising approach in measuring microcystins and nodularin is based on their inhibitory effect of PP2A and PP1 enzymes. The degree of inhibition of these enzymes can therefore be used as a measure of toxin concentration.

In this work, we propose a bi-enzyme electrochemical probe to monitor the inhibition of the enzyme PP2A by microcystin-LR and nodularin. This enzyme has a significant activity towards glycogen phosphorylase *a* (PHOS*a*), which in turn catalyses the conversion of glycogen to glucose-1-phosphate (G-1-P). The proposed system involves a preliminary phase of off–line enzymatic incubations (microcystins/PP2A, PP2A/PHOS*a*, PHOS*a*/glycogen+phosphate) followed by the electrochemical detection of H_2O_2 which is the final product of two sequential reactions catalyzed by glucose oxidase (GOD) and alkaline phosphatase (AP), co-immobilized on a H_2O_2 Pt probe inserted into a FIA system.

The total analysis time includes 50 min for the off–line enzymatic incubations and 3 min for the biosensor response.

The system calibration shows a working range of 0.5-1.3 ppb and 5-24 ppb for nodularin and microcystin-LR, respectively. These values, referred to toxin concentrations in the final assay solution, correspond to 5-13 ppb for nodularin and 50-240 ppb for microcystin-LR in water samples. For this reason, in order to assess the maximum level recommended for microcystin-LR, water samples have to be concentrated prior to the analysis. Preliminary results obtained analyzing *Planktothrix rubescens*-contaminated water samples, with a preconcentration step (using SPE Carbograph 4) will be presented. Experiments to improve the sensitivity of the method, to allow the direct analysis of water samples, are in progress.

ANA-OR-19 Total suspended solids (TSS) and polycyclic aromatic hydrocarbons (PAHs) removal from industrial/civil wastewater: a comparison between different wastewater treatments

Saer Doumett[a], Donatella Fibbi[a], Ester Coppini[b], Alessandra Cincinelli[a], Luciano Lepri[a], Leonardo Checchini[a], Massimo Del Bubba[a]

[a]Dipartimento di Chimica, Università degli Studi di Firenze, Via della Lastruccia 3, 50019 Sesto Fiorentino (FI), Italy
[b]GIDA s.p.a, Via di Baciacavallo, 36, 59100 Prato, Italy
e-mail: saer.doumett@unifi.it

In this work two wastewater treatment lines (Line 1 and Line 2) treating both industrial and domestic sewages from the west urban area of Prato and differing one from the other for the secondary settlement system, were comparatively investigated for one year. Both the treatment lines were based on conventional denitrification/nitrification systems followed in Line 1 by secondary settlement and clariflocculation tanks and in Line 2 by a membrane biological reactor. The removal of particulate material was statistically more efficient in Line 2 than in Line 1 ($P<0.01$), being mean removal percentages equal to 98.2±2.5 and 83.1±9.6, respectively. This result highlighted the very good performances of MBR for TSS removal, in agreement with literature data [1] that described a removal efficiency very close to 100% for this system and therefore much higher than the one determined for chemical clariflocculation. In any case, effluents from both the treatment lines showed TSS concentrations much lower than the legal Italian limit for discharge in surface water (35 mg l^{-1}; D.Lgs 152/2006); moreover, TSS concentrations from Line 2 were also lower than the Italian limit for wastewater reuse (10 mg l^{-1}; D.M. 185/2003), while effluent concentrations from Line 1 were often higher than this limit. PAHs with 2-3 aromatic rings were found in all the inlet wastewater samples and showed the highest concentrations, ranging from tens to hundreds of ng l^{-1}; conversely, PAHs with four to six rings were present in a smaller number of samples and at lower concentrations (from few ng l^{-1} to tens of ng l^{-1}), with the only exception of pyrene, which averaged 108 ng l^{-1}. Naphthalene represented more than 40% of total PAHs probably because of its use in the industrial production of dyes and moth repellent, both utilized in textile industry [2], whereas the priority hazardous, PAHs according to the 2455/2001/EC, represented approximately 10% of the whole PAH content. Overall PAH removal was 84±12 and 82±18 for Line 1 and Line 2, respectively, evidencing that, under the experimental conditions adopted, the two treatment lines achieved comparable performances. Removal in the particulate phase was generally higher than that in the water phase, especially for Line 2 which showed an overall PAH removal of 94% in particulate phase versus 70% in the water phase, while, for Line 1, more

similar removal percentages were observed (88 and 80% in particulate and water phases, respectively). These findings were in accordance with the results obtained for TSS. Removal percentage found for each PAH in the water phase was linearly correlated with the corresponding log K_H values, either in Line 1 (R^2=0.73; P<0.01) or in Line 2 (R^2=0.53; P<0.01), indicating that stripping phenomena play an important role in PAH removal from the water phase, especially when the more volatile compounds are considered. Mean concentrations of the six priority hazardous PAHs in outlet samples evidenced values within the environmental quality standard (EQS) of D.Lgs. 152/2006 for the sum of these compounds (0.2 μg l^{-1}) in surface waters, while the sum of Benzo(g,h,i)perylene and Indeno(1,2,3-c,d)pyrene was higher than the 2455/2001/EC EQS for inland surface waters. Benzo(a)pyrene was found to be present in 50-60% of the outlet samples, at concentrations approximately included between 10 and 30 ng l^{-1}.

[1] F.Fatone, D.Bolzonella, P.Battistoni, and F.Cecchi, *Desalination*, *183,* **2005**, 395-405.
[2] H.R. Rogers, *Sci. Tot. Environ., 185*, **1996**, 3-26.

ANA-OR-20 Electrochemical Bioassay for miRNA detection

Ilaria Palchetti[a], Serena Laschi[a], Giovanna Marrazza[a], Marco Mascini[a], Gerolama Condorelli[b], Vittorio de Franciscis[c].

[a] Dipartimento di Chimica Ugo Schiff, Università degli Studi di Firenze, Via della Lastruccia 3 50019 Sesto Fiorentino (FI), Italia
[b] Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università degli Studi di Napoli Federico II, Napoil, Napoli, Italia
[c] Istituto di Endocrinologia e Oncologia Sperimentale, CNR, Napoli, Italia
ilaria.palchetti@unifi.it

MicroRNAs (miRNAs) are naturally occurring small RNAs (approximately 22 nucleotides in length) that act as regulators of protein translation.
Because many diseases are caused by the misregulated activity of proteins, microRNAs have been implicated in a number of diseases including a broad range of cancers, heart disease, immunological and neurological diseases. Consequently, microRNAs are intensely studied as candidates for diagnostic and prognostic biomarkers. The analysis of the intracellular levels of miRNAs is challenging, however, because their short lengths, low abundances, and high levels of sequence similarity present obstacles in the use of conventional analytical methods. Currently, miRNAs are predominantly detected with Northern blot, PCR, or microarray analysis. These detection technologies are expensive and time-consuming and require well-trained scientists.

In this paper, an innovative electrochemical method based on paramagnetic beads and enzyme amplification for multiplexing miRNA detection was reported.
Magnetic beads allow easy separation and washing steps in a biosensing experimental set-up, whereas, between the different transduction principles, electrochemistry is considered one of the most appealing in term of cost, ease of use, possibility of in situ multiplexing analysis (point of care testing). The proposed method is based on biotinylated DNA CPs immobilized on streptavidn coated paramagnetic beads. Total RNA is extracted from the sample, biotinylated, and then hybridized with the beads. The beads were then incubated with streptavidin alkaline phosphatase and exposed to α-naphtyl- phosphate. The product of the enzymatic reaction was electrochemically monitored. The assay was finally tested onto a compact microfluidic platform which allows multiplexed analysis of 8 different samples.

ANA-OR-21 Analytical device based on lens-less bio-chemiluminescence imaging as a companion diagnostics tool for personalized medicine

Mara Mirasoli,[a] Luisa Stella Dolci,[a] Francesca Bonvicini,[b] Luca Cevenini,[a] Elisa Michelini,[a] Angela Buragina,[a] Massimo Guardigli,[a] Patrizia Simoni,[c] Aldo Roda[a]

[a]Dept. of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126, Bologna, Italy
[b]Dept. of Haematology and Oncological Sciences "L. e A. Seragnoli", Microbiology Section, University of Bologna, via Massarenti 9, 40138 Bologna
[c]Dept. of Clinical Medicine, University of Bologna, via Massarenti 9, 40138 Bologna
mara.mirasoli@unibo.it

Companion diagnostics are assays intended to assist physicians in making treatment decisions for their patients, by elucidating the efficacy and/or safety of a specific drug for a targeted patient group. With this respect, multiplexed Point-Of-Care Testing (POCT) devices, suitable to perform the analysis directly where the sample is obtained, are requested in order to perform rapid and accurate disease progression monitoring along with drug levels assessment.

This work describes the development of POCT devices exploiting "contact" lens-less imaging detection, in which the bio-chemiluminescence (BL-CL) analytical signal is produced directly on the surface of a CCD light sensor. This configuration provides high light collection efficiency and has been exploited in different bioanalytical assay formats [1,2]. A CL microfluidics-based device was developed for performing panel tests, including enzyme activity assays, immunoassays and nucleic acid hybridization assays, which were performed simultaneously to obtain a complete panel assay. Adequate LODs were obtained for a panel of model analytes, such as alkaline phosphatase (10 IU/L), proteins analyzed by immunoassay (3.5 fmol/L of HRP), and nucleic acids (0.05 μmol/L of amplified Parvovirus B19 target DNA). A biodevice based on BL whole-cell biosensors was developed for multiplexed detection of compounds with hormone-like activity. Cells, which were genetically engineered to express the BL reporter protein luciferase upon interaction with analytes able to activate a specific receptor, were immobilized in a modified clear bottom black 384-well microplate to obtain a BL cell array. A LOD of 0.5 nM of testosterone was obtained with immobilized yeast cell-based biosensor for androgen detection.

[1] A.Roda, M.Mirasoli, L.S.Dolci, A.Buragina, F.Bonvicini, P.Simoni, M.Guardigli, *Anal.Chem., 83*, **2011**, 3178.
[2] A.Roda, L.Cevenini, E.Michelini, B.R.Branchini, *Biosens.Bioelectron., 26*, **2011**, 3647.
This work was supported by the Italian Ministry of Instruction, University and Research (PRIN 2007 Project: 2007AWK85F_001).

ANA-OR-22 Stacking Interactions in Oligonucleotides by Differential Pulse Voltammetry and Spectroelectrochemistry Measurements

T. Caruso[a], A. Capobianco[a] , A. Peluso[a]

[a] Department of Chemistry and Biology, University of Salerno, Via Ponte don Melillo, 84084, Fisciano (SA), Italy
tcaruso@unisa.it

Oxidative DNA damage is a consequence of cellular metabolism, with a propensity for increased levels following exposure to UV and ionizing radiation, and toxic insults. One electron oxidation of DNA generates a radical cation, an electron hole, which is able to migrate along the strand and to irreversibly react leading to strand breaks and nucleobase modifications, with loss or corruption of genetic information, possibly resulting in cellular aging or disease.

Differential pulse voltammetry and spectroelectrochemistry proved to be very effective techniques to investigate the oxidation properties of isolated nucleosides and nucleotides in solution. [1-3] Here we present the results concerning the extension of our previous works to oligonucleotides, systems which are better biomimetic DNA models than single nucleosides, allowing molecular processes of free radical reactions to be examined in a less complex environment than DNA, but respectful of its biological characteristics. Short sequences (hexamers), which possess coiled conformations in water solution, have been considered. The higher order structures of the oligonucleotides have been studied by one- and two-dimensional NMR spectroscopy, circular dichroism, and molecular mechanics. Differential pulse voltammetry and spectroelectrochemistry have allowed to characterize the distribution of low lying energy states of one electron oxidized oligonucleotides, giving access to a series of important information about the chemico-physical effects which controls the long range charge transfer in DNA and determines the sites where oxidative DNA damages occur.

[1] T. Caruso, M. Carotenuto, E. Vasca, A. Peluso, *J. Am. Chem. Soc.*, *127*, **2005**, 15040.
[2] T. Caruso, A. Capobianco, A. Peluso, *J. Am. Chem. Soc.*, *129*, **2007**, 15347.
[3] A. Capobianco, M. Carotenuto, T. Caruso, A. Peluso, *Angew. Chem. Int. Ed.*, *48*, **2009**, 9526.

ANA-OR-23 Characterization of the Fat-soluble Vitamin and Carotenoid Profile of Green and Golden Kiwi by HPLC-DAD-Tandem MS Hyphenation

Simona Bellantea, Alessandra Gentilia, Fulvia Carettia, Giuseppe D'Ascenzoa, Roberta Curinia
aDipartimento di Chimica dell'Università di Roma "La Sapienza", P.le Aldo Moro 5, 00185, Rome, Italy
e-mail: simona.bellante@uniroma1.it

Fat-soluble vitamins are essential micronutrients involved in important biological functions and classified into four groups: vitamin A, vitamin D, vitamin E, and vitamin K [1]. Each group is characterized by an heterogeneity of forms about whose natural distribution little is known. In fact, the conventional analytical methods are often addressed to determine one single form, i.e. the most stable and/or widespread one. The reasons of this choice are basically economic, but also due to a series of analytical difficulties: 1) subtle chemical differences between vitamers belonging to the same vitamin group; 2) unavailability of standards; 3) low and different endogenous levels in complex food matrices; 4) occurrence of bound forms.

The main purpose of this work was to characterize the fat-soluble vitamin and carotenoid fraction of kiwifruits belonging to the genuses *Actinidia deliciosa* (green kiwi whose Italy is the world leader producer) and *Actinidia chinensis* (golden kiwi, launched on the worldwide market under the trade name Zespri Gold in 2000). A novel analytical approach, based on LC-DAD-APCI-MS/MS hyphenation, was developed in order to perform both the quantitative analysis of ten target micronutrients (lutein, zeaxanthin, β-carotene, β-cryptoxanthin, α-tocopherol, δ-tocopherol, γ-tocopherol, ergocalciferol, phylloquinone, menaquinone-4) and the screening analysis of other pigments whose standards are commercially unavailable. MSPD was used as a mild technique for the extraction/clean-up procedure, with recoveries of all compounds exceeding 60%. Non aqueous reversed phase (NARP) chromatography on a C30 column was used for the analytes separation. The combined DAD-MS chromatographic detection proved itself as a potent tool for obtaining a comprehensive profile of fat-soluble vitamers and carotenoids occurring in the analyzed fruits. The unexpected presence of menaquinone-4 (K2 vitamer) and the detection of geometric isomers, which were not artefacts of the applied extraction procedure, were only some of the achieved results.

[1] G.F.M.Ball, Vitamins in Foods: Analysis, Bioavailability and Stability, CRC Press Inc., Boca Raton, FL, **2006**.

ANA-OR-24 Determination of collagen by pyrolysis/GC-MS. Evaluation of the degree of conservation of archeological bones from Vicenne (Italy) by comparison with XRD, TGA and FTIR analysis

A. Adamiano[a], D. Fabbri[ab], G. Falini[ab], M. G. Belcastro[c]

a. CIRSA, Università di Bologna, via S.Alberto 163, 48123 Ravenna
b. Dip. Chimica G. Ciamician, Università di Bologna, 40126 Bologna
c. Dip. BES,Università di Bologna, 40126 Bologna
alessio.adamiano2@unibo.it

Human bones and teeth are frequently recovered at archaeological sites. Their state of preservation may depend on the mode and the burial environment. The content of collagen and the degree of crystallinity of carbonate hydroxyapatite (HA) are among the indicators adopted to evaluate the conservation status of bones. Analytical pyrolysis (Py) [1] together with X-ray powder diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA), were used at this scope. In this work, a new quantitative procedure in Py was employed to characterise residual proteins in five bone samples from the medieval necropolis of Vicenne-Campochiaro (Molise, Italy)[2].
The yields of cyclic dipeptides (2,5-diketopiperazines, DKPs) evolved form the pyrolysis of the samples, including the cyclo(proline-hydoxyproline) as distinctive marker of collagen, were determined by GC-MS with and without silylation. The detection of DKPs enabled the identification of collagen in all the analysed samples, in accordance to the FTIR spectra showing the characteristic amide peak. The presence of organic matter along with that of carbonatic phases was confirmed and estimated by TGA. XRD data showed that the samples mainly contained HA having different degrees of crystallinity; small amounts of quartz and calcite were also detected in some samples. The quantitative experimental data were combined to provide a relative estimate of the degree of conservation of the bone samples. The bones of an adult young female (t.139) and an aged male (t.165) resulted to be the worst and best preserved, respectively. The tombs were located in the same area where the acidity of the soil has damaged nearly all the skeletons. The skeleton from t.165 was almost complete, whereas the one from t.139 was lacking in many bones. Therefore biological (age-at-death, sex) and ritual (care, depth) factors as well as specific conditions of each burial could be involved in the observed different preservation state.

[1] B.A. Stankiewicz1, J.C. Hutchins, R. Thomson, D.E.G. Briggs and R.P. Evershed *Rapid Communications in Mass Spectrometry*, 11 (1997) 1884–1890.
[2] M.G. Belcastro, E. Rastelli, V, Mariotti, F, Facchini, B. Bonfiglioli. Continuity or discontinuity of the life-style in central Italy during the Roman Imperial Age-Early Middle Ages transition: diet, health and behaviour, *American Journal of Physical Anthropology*, 132 (2007) 381-94.

ANA-OR-25 An Alternative Method for Microcystins Analysis in Aerosol Samples.

Andrea Gambaro (1,2), Roberta Zangrando (1), Elena Barbaro (2), Elisa Scalabrin (2), Valentina Felin (2), Carlo Barbante(1,2).

(1) DAIS, University of Venice, Ca' Foscari, Calle Larga Santa Marta 2137, 30123, Venice, Italy

(2) Institute for the Dynamics of Environmental Processes CNR, Dorsoduro 2137, 30123, Venice, Italy.

gambaro@unive.it

Cyanobacteria are a small group of photosynthetic planktonic bacteria, which produce a large group of potent hepatotoxins called microcystins (MCs). Waterborne toxins can be found in the aerosol phase due to bubble-bursting processes. It has been demonstrated that some respiratory symptoms can be associated with exposure to high levels of cyanobacteria during recreational activities.

While biological assay or physicochemical approaches can reveal the presence of MCs, but not identify which specific toxins are present, chromatographic methods can separate them allowing individual identification.

The aim of this study was to obtain a sensitive method for the determination of trace concentrations of individual cyanotoxins in aerosol samples, using an Agilent 1100 series HPLC system (Agilent, Waldbronn, Germany) coupled to an API 4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Toronto, Ontario, Canada).

During method development improved electrospray ionization was found in negative ion mode for the MCs. For this reason, in contrast with others authors, we have developed a chromatographic separation using alkaline conditions, thus achieving good resolution, improved electrospray ionization and therefore better sensitivity.

A sensitive analytical method has been obtained for measuring trace concentrations of MC family of cyanotoxins and nodularin in aerosol samples, allowing a simultaneous detection of six MCs (MC-LA, -LY, -YR, -LR, -LW, -LF, and NOD) in a single 27 min chromatographic analysis.

The limit of detection for all the toxins were determined to be between 2 fg/μL (-LA and -LF) and 178 fg/μL (NOD), values that are similar or lower than those reported in the literature. In this work the internal standard method has been used for calibration and the analytical procedure was validated by evaluating the accuracy, precision and recovery .

The method was applied to seven aerosol samples from the Venice Lagoon. In these samples, trace concentrations of MC-LA ranged between 90 fg m^{-3} and 706 fg m^{-3}, MC-LF between n.d. - 369 fg m^{-3} and MC-LW between n.d. - 262 fg m^{-3}. More research needs to be conducted in order to investigate the origin of these compounds in the Venetian atmosphere.

ANA-OR-26 Assessment of the direct exposure of honeybees to particulate matter containing neonicotinoid insecticides during the corn sowing and its relevance to the colony loss phenomena.

Chiara Giorio[a], Andrea Tapparo[a], Matteo Marzaro[b], Vincenzo Girolami[b], Daniele Marton[a], Lidia Soldà[a], Alessandro Zanella[a]

[a] Dipartimento di Scienze Chimiche, Università degli Studi di Padova, via Marzolo 1, 35131 Padova, Italy
[b] Dipartimento di Agronomia Ambientale e Produzioni Vegetali, Università degli Studi di Padova, Agripolis, 35020 Legnaro (Padova), Italy
chiara.giorio@unipd.it

The last decade was characterized by rapid disappearances of honeybees' colonies failing to return to the hives. The phenomenon, called Colony Collapse Disorder (CCD), represents a worldwide crisis with consequences both in plants pollination and crops productivity. Although on the causes of CCD several hypothesis have been advanced (parasitic mites, viruses, insecticides etc.), none of them was clearly supported or refused by experimental results. Colonies losses have been reported in Italy concurrent with the sowing of corn seeds coated with neonicotinoid insecticides using pneumatic drilling machines [1]. During the sowing operations, the seeds are sucked into the pneumatic drilling machine causing the erosion of the seed coating and the fragments are then expelled through a waste pipe. In a first hypothesis, bees uptake neonicotinoids through the nectar and pollen of the contaminated vegetation at field margins. However, the insecticides content on the surrounding vegetation was shown to be not sufficient to cause acute toxicity in foraging bees [2]. In this connection, novel routes of exposure and intoxication of honeybees to neonicotinoids have been proposed [3].
In the present study the direct exposure of bees to particulate matter emitted by the drilling machine during the corn sowing has been quantified. Numerous experiments were conducted in open field and accurate analytical procedures were optimized to determine both the effective emission capability of the drilling machine and the consequent uptake of the insecticide by the bees flying over the field. Test were performed using new types of seed coating, proposed in 2009 and 2010, and three different types of pneumatic drilling machines. The results of the experiments confirm the hypothesis of the relationship between the extended honeybees losses and the sowing of corn seeds coated with neonicotinoid insecticides.

[1] Greatti M, Sabatini AG, Barbattini R, Rossi S, Stravisi A, *Bull. Insectol.*, 56, **2003**, 69–72.
[2] Greatti M, Barbattini R, Stravisi A, Sabatini AG, Rossi S, *Bull. Insectol.*, 59, **2006**, 99–103.
[3] Tapparo A, Giorio C, Marzaro M, Marton D, Soldà L, Girolami V, *J. Environ. Monit.*, 13, **2011**, in press, DOI: 10.1039/C1EM10085H.

ANA-OR-27 Application of high temperature liquid chromatography coupled to inductively coupled plasma mass spectrometry for speciation analysis of environmental samples

Francisco Ardini,[a] Amanda Terol,[b] Marco Grotti,[a] José Luis Todolí[b]

[a] Dipartimento di Chimica e Chimica Industriale, Università di Genova, Via Dodecaneso 31, 16146, Genova, Italy
[b] Department of Analytical Chemistry, Nutrition and Food Sciences, University of Alicante, Carretera San Vicente del Raspeig S/N, 03690, Alicante, Spain
ardini@chimica.unige.it

The determination of arsenic is of great environmental interest because it derives from both natural and anthropogenic sources and its toxicity and bioavailability are strongly dependent on its chemical form. The inorganic compounds (e.g. arsenite, arsenate) have been found to be very toxic, while the organic compounds can be either toxic (e.g. methylarsonic and dimethylarsinic acid) or non-toxic (e.g. arsenobetaine and arsenocholine). Furthermore, arsenosugars can occur at relatively high concentrations in organisms used as human food. Hence, it can be easily understood that speciation studies are necessary for toxicological and environmental considerations, while the determination of total arsenic is insufficient for this purpose.
The hyphenation of high performance liquid chromatography (HPLC) to inductively coupled plasma mass spectrometry (ICP-MS) is a technique of choice for speciation analysis. In recent years, high temperature liquid chromatography [1] (HTLC) has emerged as a new chromatographic technique where the mobile phase is heated up to get important advantages, such as faster separations and better resolution; moreover, organic solvents can be replaced by pure water as the mobile phase, thus making HTLC cheaper, simpler, more environmental friendly and suitable to ICP-MS than conventional HPLC.
In this work we developed new analytical methods based on HTLC-ICP-MS for arsenic-speciation analysis of environmental samples. Work temperatures higher than 100 °C allowed to obtain good separations without the need to use salts or organic solvents as the mobile phase. This method was finally applied to biological samples such as Antarctic crustaceans and molluscs.

[1] T. Teutenberg, *High-Temperature Liquid Chromatography: A User's Guide for Method Development*, RSC Publishing, Cambridge, 2010

ANA-OR-28 Preparati per la medicina ayurvedica. Contenuto di metalli essenziali e di elementi potenzialmente tossici

O. Abollino[a], A. Giacomino[a], M. Malandrino[a], M.L Colombo[b], I. Zelano[a], S. Miaglia[c]
ornella.abollino@unito.it
[a]Dipartimento di Chimica Analitica dell'Università di Torino, Via Giuria 5, 10125, Torino, Italia
[b]Dipartimento di Scienze e Tecnologia del Farmaco dell'Università di Torino, Via Giuria 9, 10125, Torino, Italia
[c]Servizio Igiene Alimenti e Nutrizione, ASL CN1, Via Torino 143, 12038, Savigliano (CN)

La medicina ayurvedica ha avuto origine in India migliaia di anni fa; è tuttora ampiamente utilizzata nel Paese di origine e sta diventando sempre più diffusa nei Paesi occidentali. In letteratura sono riportati numerosi casi di intossicazione dovuti alla presenza, in alcuni preparati ayurvedici, di elevate concentrazioni di elementi quali arsenico, piombo e mercurio, che possono essere introdotti volutamente in ciascuna formulazione, seguendo i dettami della tradizione, oppure possono derivare dai trattamenti di preparazione [1].

Per questo motivo è stato determinato il contenuto di elementi (Al, As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Pb, Si and Zn) in quindici preparati per la medicina ayurvedica acquistati in India. Gli analiti sono stati scelti tenendo conto del loro ruolo di elementi essenziali e/o tossici ad elevate concentrazioni. Sono state prese in considerazione quattro famiglie di prodotti denominate Bashma, Guggulu, Parpati, Pishti. Le analisi sono state effettuate con la spettroscopia atomica di emissione o di assorbimento, a seconda dei livelli di concentrazione in gioco, previa mineralizzazione dei campioni in forno a microonde [2]. I risultati sperimentali sono stati elaborati con tecniche chemiometriche di pattern recognition, allo scopo di rilevare similitudini e differenze tra i campioni e correlazioni tra le variabili.

Sono stati calcolati i quantitativi di ciascun elemento ingeriti seguendo la posologia riportata per ciascun preparato. Tali quantitativi sono stati confrontati con valori di riferimento, quali le concentrazioni limite tollerabili dall'organismo stabilite da organismi internazionali. Si è osservato che le concentrazioni di Hg in quasi tutti i campioni e quelle di As, Cd, Cr e Pb in alcuni casi sono superiori ai limiti di accettabilità. I risultati di questa indagine confermano i rischi associati al consumo di prodotti estranei ai circuiti di controllo regolamentati dalla Comunità Europea, soprattutto se utilizzati senza il controllo di un personale competente.

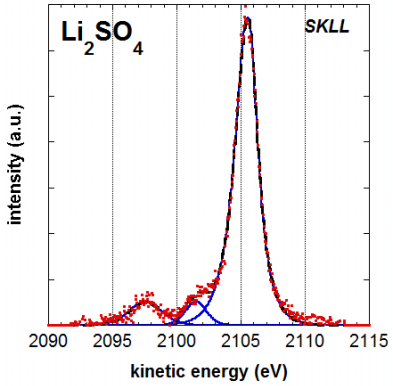
[1] R.B. Saper, R.S. Phillips, A. Sehgal, N. Khouri, R.B. Davis, J. Paquin, V. Thuppil, S.N. Kales,. JAMA 300, 2008,915.
[2] A. Giacomino, O. Abollino, M. Malandrino, K. Mani, V. Murugesan, Microchemical J., doi:10.1016/j.microc.2011.01.002.

ANA-OR-29 X-ray photoelectron spectroscopy and X-ray excited Auger electron spectroscopy of alkali metal sulphates

M. Fantauzzi, A. Rigoldi, D. Atzei, B. Elsener, A. Rossi

Department of Inorganic and Analytical Chemistry, INSTM research unit, University of Cagliari, I-09042 Monserrato (Ca), Italy
fantauzzi@unica.it

The dissolution of sulphide minerals results in acid-mine drainage with possible release of toxic elements. The surface reaction is controlled by a sulphur-containing thin film in intermediate oxidation states [1-4]; their assignment is still controversial. XPS and XAES are powerful techniques for directly probing the chemical state of the elements, but their application to these minerals has been hampered by difficulties in interpreting the small shifts in S2p binding energies in the region of 164-165 eV and in the curve fitting of the SKLL spectra. So far the sulphur Auger parameter $\alpha'=KE_{Auger}+BE_{photoelectron}$[5] was calculated considering the kinetic energy of the maximum of SKLL peak [2-4]. Here the results of the peak-fitting of the sulphur SKLL signal of alkali metal sulphates are provided. The example in the figure shows the presence of three components that can be identified as follows: the most intense, ^1D, is due to the $KL_{2,3}L_{2,3}$ transition that corresponds to the $2s^22p^4$ final configuration. The second one at lowest kinetic energies, ^1S, is due to the other possible transition, KL_1L_1, that corresponds to the $2s^02p^6$ final configuration; the third signal, between ^1D and ^1S lines, is probably an excitation line due to a satellite. This component is present in all SKLL sulphate spectra. An increase in SKLL KE was observed with increasing metal atomic number (from 2105.1 eV for Li_2SO_4 to 2107.5 eV for Cs_2SO_4) together with a decrease of S2p binding energy. The Auger parameter is fairly constant and the difference of S2p and SKLL energy values are interpreted as differences in Madelung potentials.



1. A. Rossi, D. Atzei, S. Da Pelo, F. Frau, P. Lattanzi, K.E.R. England and D.J. Vaughan *Surf. & Interf. Analysis 31*, **2001**, 465
2. M. Fantauzzi, B. Elsener, D. Atzei, P. Lattanzi and A. Rossi *Surf. & Interface Anal. 39*, **2007**, 908.
3. B. Elsener, D. Atzei, M. Fantauzzi and A. Rossi *Eur. J. Mineral* 19, **2007**, 353.
4. M. Fantauzzi, D. Atzei, B. Elsener, P. Lattanzi and A. Rossi, *Surf. & Interf. Anal.* 38, **2006**, 922.
5. C.D. Wagner e A. Joshi, *J. Electron Spectrosc. Relat. Phenom. 47*, **1991**, 283

ANA-OR-30 Determination of YLOID, Zr and Hf in seawater by ICP-MS technique: method validation and evaluation of measurement uncertainty

M. Raso,[a*], F. Saiano[b], P.Censi[a]

[a] Di.S.Te.M., Università di Palermo, Via Archirafi 22, 90123, Palermo, Italy.
[b] Dip. S.Ag.A., Università di Palermo, Viale delle Scienze ed. 4, 90128, Palermo, Italy.
rasomaria.mr@libero.it

In the last years many researches were focused on behaviour and naturally abundances of Y, La, Lanthanoids (YLOID), Zr and Hf in marine systems increasing the attention to their capability to trace geochemical processes occurring along the water column, ocean circulations and at the solid-liquid interface [1-2]. In contrast YLOID and Zr-Hf behaviour can have a geochemical significance only if their contents in seawater is analysed according to a robust, reliable and comparable chemical analytical approach. Therefore the ability of an analytical protocol to obtain reproducible values is paramount. Unfortunately, the analysis of these metals in natural waters is often complicated by their ultra low concentrations (0.5–100 pmol/L), the high matrix concentration and a wide range of severe spectral interferences (*e.g.*, Ba^{2+}, seawater salts).

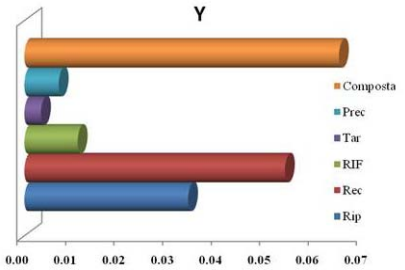


Fig. 1

To perform simultaneous ultra-trace YLOID, Zr and Hf analyses in seawater, we developed a preconcentration method based on coprecipitation with $Fe(OH)_3$ and determination by ICP-MS. In this study the metals behaviour was quantitatively investigated during coprecipitation and estimation of composed uncertainty associated to measurements was evaluated with a rigorous metrological approach based on method validation and quality data control. These goals were achieved using spiked natural seawater samples where YLOID, Zr and Hf had concentrations as occurring in natural seawater, (20 pg/mL).

Under these conditions the metals were quantitatively recovered from seawater with good precision (2–5%), apart for La (10%). Composed measurement uncertainty was expressed in terms of precision, recovery, reference materials and instrumental calibration uncertainty (Fig.1). The obtained results were critically discussed on the basis of the different contributions and confirm the quadrupole ICP-MS technique as highly sensitive to determine very low YLOID, Zr and Hf concentrations.

[1] D. S Alibo. and Y. Nozaki Deep- Sea Research, 51, **2004**, 559–576.
[2] P. Möller, P. Dulski and Luck J. Spectrochimica Acta, 47,**1992**, 1379.

ANA-OR-31 Development and analytical characterization of a novel *in situ* antibiotic delivery system to prevent titanium implant-related infections

E. De Giglio[a], G. Trapani[b], D. Cafagna[a], R. Iatta[c], S. Cometa[d], G. Camporeale[a], M. Mattioli-Belmonte[e], L. Sabbatini[a]

[a*] Dept. of Chemistry, University of Bari Aldo Moro, Italy
[b] Dept. of Pharmaceutical Chemistry, University of Bari Aldo Moro, Italy
[c] Dept. of Biomedical Sciences and Human Oncology, University of Bari Aldo Moro, Italy
[d] BIOLab, Chemistry & Industrial Chemistry Department, University of Pisa, Pisa, Italy
[e] Dept. of Molecular Pathology-Innovative Therapies, Marche Polytechnic University, Ancona, Italy

E-mail: sabba@chimica.uniba.it

Orthopedic infections often represent one of the major causes of implant failure [1]. In this context, an efficient approach is based on local antimicrobial prophylaxis generally performed by coating titanium implant surfaces with active thin films able to release antibiotic [2]. In this work, a novel Ciprofloxacin (CIP) loaded chitosan nanoparticles (CSNPs) coating onto titanium surface has been developed and characterized.
The model antibiotic CIP is active against Gram-positive and Gram-negative bacteria in vitro and it also shows high stability and efficiency [3]. On the other hand, NPs based on the cationic polysaccharide chitosan are biocompatible and their ability to load and deliver hydrophilic molecules such as CIP is well documented [4, 5].
CSNPs loading CIP were prepared according to a modified ionic gelation method [4]. Afterwards, CIP loaded CSNPS were set by casting onto titanium sheets. The determination of particle size and polydispersity index of CIP loaded CSNPs were determined using a Zetasizer NanoZS. X-ray Photoelectron Spectroscopy (XPS) analysis was performed on pure materials and on CIP loaded CSNPs system in order to provide information about the drug surface location on the coating.
Drug release profile from the investigated coatings, tested in physiological solution by HPLC, showed that, since the first hours of incubation, the CIP amount released over time is higher than the minimum inhibitory concentration (MIC) values of the most common pathogens causing orthopaedic implant infections. A study on the antibacterial activity of these nanoparticles-based coatings was performed on *S. aureus* and *P. aeruginosa* cultures, demonstrating the total inhibition growth of both bacteria.

Coatings biocompatibility was also assessed by MTT test and SEM morphological analysis using MG63 ostoblast-like cells highlighting a good cell viability. Thus, the novel antibiotic delivery system investigated represents a promising coating that could act as potential *in situ* drug carrier.

[1] Y. Qiu, N. Zhang, Y.H. An and X. Wen, *Int. J. Artif. Organs 30*, **2007**, 828.
[2] E. De Giglio, S. Cometa, M.A. Ricci, D. Cafagna, A.M. Savino, L. Sabbatini, M. Orciani , E. Ceci, L. Novello, G.M. Tantillo, M. Mattioli-Belmonte, *Acta Biomaterialia 7*, **2011**, 882.
[3] E.S.R. Darley and A.P. Mac Gowan, *J. Antimicrob. Chem. 53*, **2004**, 928.
[4] A. Trapani, M. Garcia-Fuentes and M.J. Alonso, *Nanotechnology 19*, **2008**,185101/1.
[5] E. De Giglio, A. Trapani, D. Cafagna, L. Sabbatini, S. Cometa, *Anal. Bioanal. Chem. 400*, **2011**, 1997.

ANA-OR-32 Looking at the liquid surface-vacuum interface by X-ray photoelectron spectroscopy

A.Rossi[°*] and N.D. Spencer[°]
M. Hess[§], U. K. Krieger[§], T. Peter[§]
[*]Department of Inorganic and Analytical Chemistry, INSTM research unit, University of Cagliari, I-09042 Monserrato (Ca), Italy
[°]Department of Materials, ETH Zurich, Switzerland
[§]Swiss Federal Institute of Technology (ETH), Institute for Atmospheric and Climate Science, Universitätstrasse 16, CH-8092 Zurich, Switzerland
e-mail person responsible of the presentation: rossi@unica.it

The strength or the weakness of an electrolyte in organic solvents is interesting both from the technological and the scientific point of view, e.g. in high-energy battery production and the elucidation of organic reactions mechanisms, respectively. In this work the behavior of tetraethyl-ammonium bromide (TEABr) in polyethylene glycol (PEG) 200 by means of conductimetric measurements and angle resolved X-ray photoelectron spectroscopy (ARXPS) has been investigated to verify if they are strong or weak electrolytes and to characterize the spatial distribution of the ions at the vacuum-solution interface.

Comparing our conductimetric data with Onsager's equation shows that solutions of TEABr in PEG 200 exhibit the typical behavior of strong electrolytes. The specific conductivity has also been measured for saline solutions in water and in ethylene glycol and a good agreement between the data of this work and those of the literature was found.

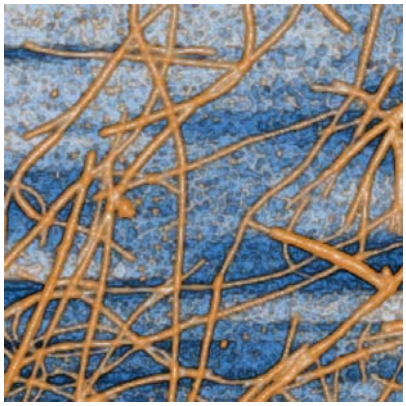
The XP-spectra of the 5wt% of TEABr in PEG 200 were taken on the liquid deposited as drop on gold. All peak positions appear to remain unchanged with respect to those of pure PEG and TEABr. ARXPS has shown that both anions and cations are repelled from a vacuum-solution interface. The thickness of the ion-depletion layer is found to be approximately 8 Å.

ANA-OR-33 Solvation effects on the supramolecular conformation adopted by an elastin-like polypeptide, polyValGlyGlyValGly. An investigation on interfacial properties by combined surface techniques.

Pasquale Moscarelli,[a] Anna Maria Salvi,[a] Brigida Bochicchio,[a] Antonietta Pepe[a] and James E. Castle[b]

[a]Dipartimento di Chimica "Antonio Mario Tamburro", Università della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy.
[b]School of Engineering, University of Surrey, Guildford GU2 7XH, Surrey, United Kingdom.
pasquale.moscarelli@hotmail.it

Previous work on elastin-like polypeptides (ELPs), made of hydrophobic amino acids, such as valine, glycine and leucine, has shown that helical fibres easily form in aqueous media [1,2] while globules and 'string of bead' structures were observed to form in deposits from methyl alcohol [3]. The supramolecular organization seems governed by local interfacial interactions and, thus, considerations of the interface properties, in the given environment, are important.



We have here focussed our attention on polyValGlyGlyValGly, chemically synthesized and characterized in our laboratories [1-3]. This polypeptide, the most inclined in forming long helical fibres in water [4], see Figure, was dissolved in solvents having different protic and polar characters (H_2O, MeOH, DMSO, EG). Based on past experience, we have then examined the deposits, evaporated onto silicon substrates, using AFM and XPS analyses to combine the images of the obtained supramolecular structures with their surface composition.

The manner in which the preferred organization of polyValGlyGlyValGly are obtained, in dependence of the environment, is discussed also in the light of the possible biological role of this polypeptide, as a support for tissue regeneration and engineering applications.

1. R. Flamia, G. Lanza, A.M. Salvi, J.E. Castle, A.M. Tamburro, *Biomacromolecules,* **2005**, 6, 1299-1309.
2. R. Flamia, A.M. Salvi, L. D'Alessio, J.E. Castle, A.M. Tamburro, *Biomacromolecules,* **2007**, 8, 128-138.
3. R. Flamia, P.A. Zhdan, M. Martino, J.E. Castle, A.M. Tamburro, *Biomacromolecules,* **2004**, 5, 1511-1518.
4. A.M. Salvi, P. Moscarelli, G. Satriano, B. Bochicchio and J.E. Castle, *Biopolymers,* | DOI: 10.1002/bip.21636 Article first published online: 20 APR **2011**.

ANA-OR-34 Reaction chromatography: design and characterization of new stationary phases for flow-chemistry applications

Alberto Cavazzini, Alessandro Massi

Dipartimento di Chimica dell'Università di Ferrara, Via L. Borsari 46, I-44121, Ferrara, Italy
alberto.cavazzini@unife.it

Organocatalysis uses small organic enantiopure molecules, usually derived from the natural world (proline, amino acids, alkaloids of the cinchona family, etc.). These molecules do not require controlled reaction conditions such as anhydrous or purified solvents or an inert atmosphere. Compared to metallocatalysis, organocatalysis has the advantage that product contamination is avoided since metals are not used. On the other hand, organocatalysts are characterized by lower catalytic efficiencies than metallocatalysts. For this reason, they are employed in higher loading, generally in the range 5-20% which has to be compared with 0.01-1% usually employed for metal based catalysts.
The immobilization of reagents or catalysts on insoluble solid supports (such as silica gel) enables the synthetic processes to occur in heterogeneous phase, a situation that shows clear advantages compared to homogeneous systems. In fact, the simple isolation of the catalyst and its potential recycling make the solid-supported catalyst processes particularly attractive in many fields of fine chemicals (included that of asymmetric synthesis of chiral enantiopure products). Heterogeneous catalysis performances are strictly depending on both the nature of support and the type of surface immobilization of catalytically active fragments. The support should ensure high thermal, chemical and mechanical stability together with high surface area, thus enabling rapid mass transfer for reagents, products and catalysts. In this work, we describe the preparation and the chromatographic characterization of new materials prepared by covalently binding proline and proline-like organocatalysts to silica gel. These materials can be used in chromatography as chiral stationary phases or in chromatographic-like applications as supports to perform chemical reaction in flow-mode.
In particular, the slow aldol reaction of cyclohexanone with p-nitro benzaldehyde has been selected as model reactions to study the activity and stability of these materials under continuous-flow conditions.

ANA-OR-35 Effect of temperature on packing and performance of nano-LC columns.

Fabiana Capriotti, Pierangela Palma, Irene Leonardis and Achille Cappiello,

Dipartimento di Scienze della Terra, della Vita e dell'Ambiente (DiSTeVA) – Laboratorio LC-MS - Università degli Studi di Urbino "Carlo Bo", Piazza Rinascimento 6, 61029, Urbino, Italy
f.capriotti@hotmail.it

Over the years, much effort has been addressed to increase the speed of analysis and the efficiency in HPLC. Nano-LC columns allow to work with minute sample size and small volumetric flow-rates, therefore, they show an increase of sensitivity due to a reduced sample dilution. Concentration-sensitive detectors, such as electrospray mass spectrometry, can take advantage of this increase when high sensitivity is required.

In this work, we evaluated the effect of temperature on the packing procedure of nano-LC columns (75 μm i.d.) and on their performance.

Different slurries of packing material were prepared using different solvent mixtures, and the stability of the suspension was evaluated at different temperatures. At high temperature (70° C) the slurry sedimentation is slow and the suspension looks stable for, at least, 30 minutes. As a consequence, at high temperature it was possible to easily pack long and more efficient columns (50 cm and longer). Long (40-50 cm) and short (15-20 cm) columns were packed at room temperature and at 70° C, using three different slurries. For long columns, a lab-made end-frit was syntherized directly in the fused silica capillary tubing. The empty tubing was packed with C18 Pinnacle II stationary phase (Restek, Bellefonte, PA, USA). For short columns, used Integrafrit fused silica capillary tubing was used and packed with Poroshell 120 SB-C18 stationary phase (courtesy of Agilent Technologies, Santa Clara, CA, USA). Long columns were tested at 70°C to reduce the mobile phase viscosity; short columns were tested at room temperature. The performance of the columns was evaluated through the calculation of the following parameters: capacity factor, k; asymmetry factor, As; number of theoretical plates, N; Van Deemter with reduced parameters plot [1]. A test mixture containing uracil (t0), benzene, naphthalene, and biphenyl (Restek, Bellefonte, PA, USA) was used to evaluate the chromatographic parameters.

The columns packed at a high temperature show a better efficiency, in terms of theoretical plates, that those packed at room temperature, depending on the slurry composition, with both stationary phases. The asymmetry factor is worse for the Poroshell packed columns, with all three slurries, compared to C18 Pinnacle II. The unsatisfactory As values can indicate that the Poroshell stationary phase deteriorates at 70°C.

ANA-OR-36 Retinoids in Raw Milk from Different Animal Species: a Complete Analytical Strategy Based on LC-MS/MS Hyphenation

Fulvia Caretti[a], Alessandra Gentili[a], Roberta Curini[a], Giuseppe D'Ascenzo[a], Stefano Marchese[a], Silvia Rocchi[a]
[a] Dipartimento di Chimica dell'Università di Roma "La Sapienza", P.le Aldo Moro 5, 00185, Rome, Italy
fulvia.caretti@uniroma1.it

Milk is an almost complete single food containing significant amounts of essential nutrients; its whey is a good source of water-soluble vitamins, while its lipid fraction is an important delivery medium of fat-soluble vitamins, especially vitamin A [1]. Vitamin A-active compounds occur in milk mainly as retinoids and to a lesser extent as carotenoid precursors (provitamins A).

Very little is known about the quali-quantitative profile of retinoids in bovine milk as well as in that of other ruminant species. Only one study [2], describes the HPLC-UV analysis of retinyl esters in cow, goat and human milk. The main analytical difficulties are related to the unavailability of standards, their cost and the complexity in development chromatographic separation; in fact, vitamin A vitamers are characterized by subtle differences in chemical structures so a highly efficient and selective chromatographic system is needed for achieving their resolution.

A reliable analytical approach, based on high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS), allowed us to establish the real occurrence and distribution of several retinoids in raw cow, sheep, goat and water buffalo milk samples. Direct liquid extraction with solvents was performed for isolating retinoids while alkaline hydrolysis enabled the total retinol determination; yields exceeding 68% were obtained for all analytes. Chromatographic separation was carried out using two tandem systems of reversed-phase columns (C18/C18 and C18/C30) in order to achieve total separation of the vitamin A vitamers. The chromatograph was coupled on-line with a triple quadrupole mass spectrometer, and the MS detection was accomplished by means of positive atmospheric pressure chemical ionization (APCI), operating in the Selected Reaction Monitoring (SRM) mode.

According to our results, and in the light of the FAO/WHO recommendations [3], the consumption of milk may supply a significant portion of the daily intake of vitamin A, proving to be an important food, ***especially for infants and children.*** Buffalo milk, in particular, has a high level of retinyl linolenate, that represents an additional nutritional value, since omega-3 fatty acids are essential for the proper functioning of the organism.

[1]G.F.M.Ball(ed),Vitamins in Foods.Analysis, Bioavailability, and Stability, **2006**.
[2]D.C.Woollard, H.Indyk, *J. Micronutr. Anal.*, **1989**, 5, 35.
[3] http://whqlibdoc.who.int/publications/2004/9241546123.pdf

ANA-OR-37 Ionic liquids as novel coatings for solid-phase microextraction

F. Bianchi, F. Bisceglie, M. Careri, A. Mangia

Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Università degli Studi di Parma, 43124 Parma
E-mail: federica.bianchi@unipr.it

Ionic liquids (ILs) are molten salts made of cations and anions having a melting point below 100 °C [1]. Beside the intrinsic non-molecular nature of ILs giving them unique solvent properties, the major advantage is their extremely low vapor pressure. Some properties, such as thermal stability and miscibility, mainly depend on the anion, whereas others, such as viscosity, surface tension and density, depend on the length of the alkyl chain in the cation. By a proper combination of anions and cations, different ILs having desired chemico-physical properties can be obtained. In a research program aimed at devising novel SPME coatings for forensic applications [2], the aim of this study was the development of novel IL fibers for solid-phase microextraction (SPME) of drugs of abuse. Four polymeric ILs (Fig. 1) were synthesized and used for the environmental monitoring of drugs of abuse with the aim of detecting both consumption and their illicit import.

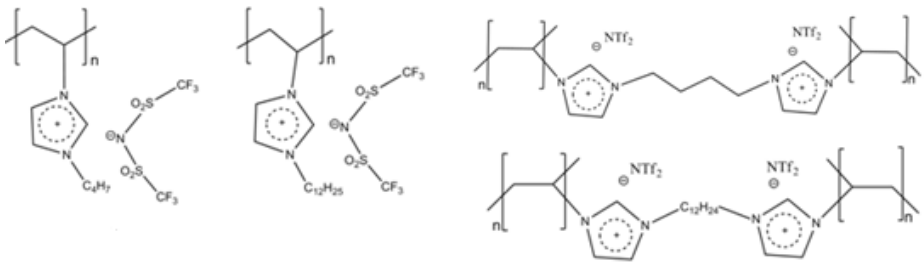


Figure 1

The SPME fibers were characterized in terms of coating thickness, thermal stability, bleeding and pH stability obtaining extraction capabilities higher than those obtained using commercial devices.
Method validation proved the reliability of the developed SPME-GC-MS method for the environmental detection of drugs of abuse at trace levels. Finally, the method was applied to the analysis of seized drapery showing the presence of ketamine at high concentration levels.

[1] A. Berthod, M.J. Ruiz-Angel, S. Carda-Broch, J. Chromatogr. A, 1184 (2008) 6
[2] M. Mattarozzi, F. Bianchi, F. Bisceglie, M. Careri, A. Gregori, A. Mangia, Anal. Bioanal. Chem., 399 (2011) 2741

ANA-OR-38 Pressurized solvent extraction for the determination of illicit drugs in hair by HPLC-MS/MS

Manuel Sergi[a], Sabino Napoletano[b], Roberto Iofrida[b], Camilla Montesano[b], Dario Compagnone[a], Roberta Curini[b], Giuseppe D'Ascenzo[b]

[a] Department of Food Science, University of Teramo, Via Carlo Lerici 1, 64023 Mosciano Stazione (TE), Italy y
[b] Department of Chemistry, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Ital
e-mail: msergi@unite.it; sabino.napoletano@uniroma1.it

Plasma and urine are the most commonly used matrices for illicit drug testing, but since 1979 there has been an increasing interest in the development of analytical methods in alternative matrices such as hair [1]. Hair has the advantage of a substantially longer detection window (months to years) which enables retrospective investigation of chronic consumption. In addition, hair is a durable and stable matrix difficult to adulteration in which toxic substances are pre-concentrated and remain for a long time without significant alterations; furthermore the sampling is not invasive [2]. Indeed hair analysis of illicit drugs has recently been codified in the Italian legislation as a monitoring tool in the field of workplace safety [3]. The critical step is certainly the extraction phase, usually performed by solid liquid extraction for multiclass methods; the main drawback is the long contact time (from 16 to 20 hours) between hair and solvent necessary to have good recoveries. Times are greatly reduced if the extraction is assisted by ultrasonic or increased temperature.

Pressurized Liquid Extraction (PLE) was applied to significantly reduce extraction time since there are no PLE applications in this field. So a method based on HPLC-ESI-MS/MS has been developed and validated for multiresidual determination of illicit drugs from hair: amphetamine, methamphetamine, mescaline, MDA, MDMA, MDEA, cocaine, benzoylecgonine, nor-cocaine, ketamine, phencyclidine, diacetylmorphine, morphine, 6-monoacetylmorphine, codeine. PLE led to reduce the analysis time and automate the extraction process, taking into account sample washing to remove external contamination and the clean-up of the extracts. Satisfactory extraction rates have been obtained using water as extracting and 10 minutes as extraction time.

[1] M. Wada et al., *Anal.Bioanal.Chem.*, 397, **2010**, 1039.
[2] F. Pragst et al., *Clin.Chim.Acta*, 370, **2006**,17.
[3] Accordo Stato/Regioni 18.09.**2008** (G.U. n. 236 del 08.10.08)

ANA-OR-39 Optimization of the Separation of Biomolecules by Capillary Electrophoresis and High Performance Liquid Chromatography: Effects of the Liquid Phase Composition

Danilo Corradini, Antonella De Rossi, Isabella Nicoletti

Istituto di Metodologie Chimiche del CNR, Area della Ricerca di Roma 1, Via Salaria Km 29,300 Montelibretti, C.P. 10, 00016 Monterotondo Stazione (Roma)
E-mail: danilo.corradini@imc.cnr.it

This communication discusses the results of our recent studies carried out to shed light on theoretical and practical aspects of capillary electrophoresis (CE) and high performance liquid chromatography (HPLC), which are widely employed analytical techniques for the separation and identification of biomolecules in natural complex matrices [1]. We examine the influence of the composition of the liquid phase (i.e. the electrolyte solution (BGE) in CE and the mobile phase in HPLC) on the electrophoretic and chromatographic behaviour of several classes of biological compounds, which are typically analysed by CE or/and by HPLC, either with UV-Vis or mass spectrometry (MS) detection. In addition, we discuss the successful employment of CE to investigate the occurrence of interactions between the analytes and the components of the solutions used as the mobile phases in HPLC.

The research has been carried out investigating mobile phases and BGEs of composition requested to control the protonic equilibrium in solution and to modulate the selective separation of peptides, proteins and other biomolecules either by CE or by HPLC. Variations of the mobile phase composition in a wide range of ionic strength determine significant differences in the chromatographic behaviour of peptides and proteins on size exclusion and ion-exchange silica based HPLC columns, which have been related to the capability of the investigated analytes to establish electrostatic and hydrophobic interactions with the above columns. The interactions of peptides and proteins with the acidic components of the mobile phases employed for their separation by reversed phase HPLC have been confirmed by CE and their effects on the chromatographic behaviour of these analytes are discussed. Also examined is the use of buffering agents capable to controlling the protonic equilibrium in a wide pH range and the use of additives incorporated into the BGE to suppress the untoward interactions of basic proteins with the inner surface of the bare fused-silica capillary employed in CE [2-3].

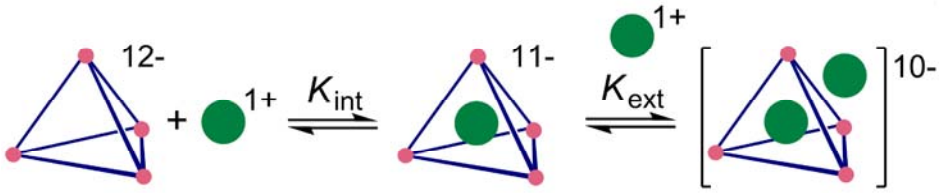
[1]Handbook of HPLC Second Edition, D. Corradini (Ed.), CRC Press, Boca Raton (USA), 2010.

[2]S. Bachmann, R. Vallant, R. Bakry, C.W. Huck, D. Corradini, G.K. Bonn, Electrophoresis 31, **2010**, 618-629.

ANA-OR-40 Thermodynamics of a Supramolecular Host in Water: Deconvoluting the External and Internal Guest Binding

Carmelo Sgarlata,[a] Giuseppe Arena,[a] Kenneth N. Raymond[b]
[a] Dipartimento di Scienze Chimiche, Università degli Studi di Catania, Catania, Italy;
[b] Department of Chemistry, University of California, Berkeley, CA and Chemical Science Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA
sgarlata@unict.it

The supramolecular assembly $[Ga_4L_6]^{12-}$ (L = 1,5-bis(2,3-dihydroxybenzamido)naphthalene) has been reported to act as a chiral, nanoscale flask suitable to mediate the reactivity of encapsulated reactive guests and to carry out enzyme-like chemical transformations [1]. The highly anionic exterior surface of the assembly imparts solubility in water and other polar solvents and affinity for the external ion-association of cationic molecules [2]. The driving forces for the external and internal guest binding are very different thus complicating the determination of the thermodynamic parameters. We have used a combination of NMR, UV-vis and isothermal titration calorimetry to definitively separate multiple guest binding to the interior and exterior of the supramolecular host and to determine the corresponding $\Delta G°$, $\Delta H°$ and $\Delta S°$ values [3]. Data obtained by each independent technique measure different components of the host-guest equilibria and only when analyzed together and simultaneously a complete picture of the solution thermodynamics emerges. Striking differences between the internal and external binding of ammonium guests are found as a consequence of the high charge and hydrophilic outer space of the host contrasted by its hydrophobic inner space.

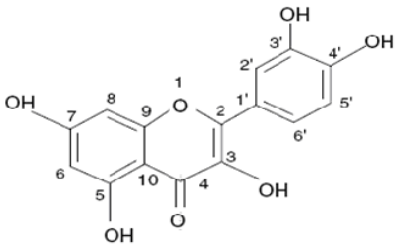


[1] M. D. Pluth, R. G. Bergman and K. N. Raymond, *Acc. Chem. Res.*, *42*, **2009**, 1650.
[2] M. D. Pluth, D. W. Johnson, G. S. Szigethy, A. V. Davis, S. J. Teat, A. G. Oliver, R. G. Bergman and K. N. Raymond, *Inorg. Chem.*, *48*, **2009**, 111.
[3] C. Sgarlata, J. S. Mugridge, M. D. Pluth, B. E. F. Tiedemann, V. Zito, G. Arena and K. N. Raymond, *J. Am. Chem. Soc.*, *132*, **2010**, 1005.

ANA-OR-41 Lanthanides(III) compounds with quercetin

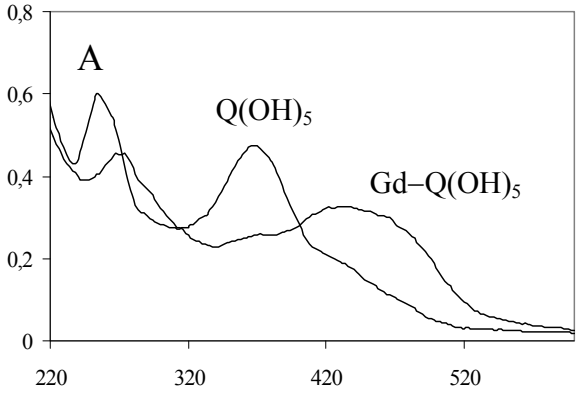
G. De Tommaso[a], T. Caruso[b], M. Iuliano [a], E. Vasca[b]

[a] Dipartimento di Chimica dell'Università degli studi di Napoli "Federico II", Via Cinthia, 45, 80126, Naples, Italy.
[b] Dipartimento di Chimica dell'Università degli studi di Salerno, via Ponte don Melillo
84084, Fisciano (SA), Italy.
gaetano.detommaso@unina.it



Quercetin (3, 3', 4', 5, 7 – pentahydroxyflavone), $Q(OH)_5$, belongs to the group of flavonoids, a class of polyphenols, found in high concentrations in a wide variety of plants. Flavonoids have been associated with prevention of heart disease and cancer. Quercetin is the most abundant of the flavonoid molecules and exhibits strong antioxidant properties and inhibitory effects on several enzymes. These properties are ascribable to their abilities to act as chelators to metal ions. Recent studies have, in fact, highlighted the possibility of using quercetin as a binder to reduce the toxicity of many metals[1].

quercetin = $Q(OH)_5$



The complexation of lanthanoids(III) with quercetin has been evidenced by spectroscopic and potentiometric techniques. The system Gd(III)–quercetin has particular relevance in medical imaging. In patients with diabetes and kidney dysfunction, the Gd^{3+} is not well tolerated[2]. However, the administration of complexed gadolinium will reduce their toxicity.

The aim of this work is the study, at 25°C and 0.5 mol/dm^3 NaCl by potentiometric (glass electrode) and spectrophotometric titrations, of the complex formation between quercetin and H^+ as well as between Gd^{3+} ion and quercetin in H_2O–C_2H_5OH 5%(w/w) solutions. The presence of ethanol has been necessary to increase the quercetin solubility due to its low solubility in water (4×10^{-6} mol/dm^3).
The data were processed by numeric methods (HYPERQUAD2008) and the results are explained by the equilibria and constants.

Equilibria	EMF data log$K \pm 3\sigma$	SPECT data log$K \pm 3\sigma$
$Q(OH)_5 \rightleftharpoons Q(OH)_4O^- + H^+$	–5.6 ± 0.1	–5.46 ± 0.05
$Q(OH)_5 \rightleftharpoons Q(OH)_3O_2^{2-} + 2H^+$	–13.2 ± 0.2	–12.93 ± 0.08
$Q(OH)_5 \rightleftharpoons Q(OH)_2O_3^{3-} + 3H^+$	–21.2 ± 0.2	–21.0 ± 0.1
$Gd^{3+} + Q(OH)_5 \rightleftharpoons GdQ(OH)_3O_2^+ + 2H^+$	–2.5 ± 0.1	–2.48 ± 0.05

[1] J. Zhou, L. Wang, J. Wang, N. Tang, *Journal of Inorganic Biochemistry,* 2001,83,41–48.
[2] RY. Kwong et al., *Circulation*, 2008,118,1011–1020.

ANA-OR-42 Constants of slight soluble acids. Determination by Coulometry

Marco Antonilli, Emilio Bottari, Maria Rosa Festa, Lorella Gentile
Dipartimento di Chimica, ed. Cannizzaro, Università "La Sapienza", Piazzale A. Moro 5, 00185 Roma
e-mail: emilio.bottari@uniroma1.it

The flowing of constant direct current allows planning a coulometric method to determine solubility and acidity constants of slight soluble acids. Coulometry and electromotive force measurements are applied to obtain such parameters for eight bile acids in solutions without formation of micellar aggregates. This method can be applied to little soluble acids without complicated elaboration of the experimental data.

This communication is applying it to cholic (HCol, (3α,7α,12α-trihydroxycholanic), glycocholic (HGC), deoxycholic (HDC, 3α,12α-dihydroxycholanic), glycodeoxycholic (HGDC), chenodeoxycholic (HCDC, 3α-7α dihydroxycholanic), ursodeoxycholic (HUDC, 3α,7β-dihydroxycholanic) lythocholic (HLC, 3α-hydroxycholanic) and dehydrocholic (HDHC, 3α,7α,12α-trihyketholanic) acids.

Solubility and acid constants are determined at 25°C and in constant ionic medium 1.00, 0.50 and 0.15 mol dm^{-3} NaCl. The values determined in 0.15 mol dm^{-3} NaCl are directly applicable (in physiologic conditions). Also the others have thermodynamic values. The method of the ionic medium [1], minimizing the variation of the reagent activity coefficients in spite of the change of their concentration, allows to substitute activities with concentrations.

To determinate solubility, *s*, an excess of each acid is added to an aqueous solution of the selected ionic medium stirring until equilibrium was reached. The saturated solutions are filtered (Solutions S') and potentiometrically titrated by means of the following galvanic cell: R.E. /Solution S' / G.E. (I)

The titration of solution S' is carried out coulometrically. The equivalence point was appreciated applying a modification to the method proposed by Gran [2].

The equilibrium: $HA \Leftrightarrow H^+ + A^-$, is defined by the constant: $k = c_H\, c_A / c_{HA}$ (1).

The free concentration c_{HA} in equilibrium with the corresponding solid is constant (i.e. HA solubility, *s*). Eq. (1) can be written: $c_{HA}\, k = K' = c_H\, c_A$ (2), where K' is constant. Electromotive force (e.m.f.) measurements of the galvanic cell:

R.E. /solution S/ G.E., (II) provides c_H.

The solution was gradually alkalinised generating constant current by coulometry. The e.m.f. measurement and the electricity quantity allow to calculate K'of eq. (2) for each addition of current. Combining K' values with solubility, *s*, acid constants k for each HA are calculated.

[1] G. Biedermann and L.G. Sillèn, *Arkiv Kemi*, *5*, **1953**, 425.
[2] G. Gran, *Analyst*, *77*, **1952,** 661.

ANA-OR-43 Analytical performances of bioluminescence cell-based portable biosensors for on-site multiplex applications.

A.Roda

Laboratory of Analytical and Bioanalytical Chemistry, Department of Pharmaceutical Sciences, Alma Mater Studiorum-University of Bologna, via Belmeloro 6, 40123, Bologna, Italy

Genetically engineered cells such as bacteria, yeasts, or mammalian cells able to produce a specific bioluminescent (BL) analytical signal in response to a target analyte represent a potent analytical tools for environmental toxicity, medical, and food analysis, being characterized by potential low cost and high content screening and multiplex applications [1].
Nevertheless some important issues such as portability and reliability related to the use of "live" cells have still to be addressed to make these biosensing devices true analytical biosensors giving precise and accurate quantitative information. Indeed, their main pitfall is the high variability of the BL signal produced by the engineered cell, in fact the emitted light changes according to the metabolic state of the cell. To solve this problem we introduced in the cell a vitality internal BL control to correct the analytical signal [2].
In the same cell, we introduced two firefly luciferases mutants requiring the same luciferin substrate emitting at different wavelengths, green and red, which are spectrally resolved. The expression of one was analyte specific and the other, constitutively expressed, was used as a cell viability internal control.
Cells were then immobilized in a polymeric matrix composed of an aqueous mixture of agarose, PVP and collagen ensuring their long term viability .
A device was constructed with the cell array in contact, through a fiber optical taper, with an imaging light sensor, a portable charge-coupled device (CCD) camera able to localize and quantify the luminescent signal. Lensless light detection, in which the signal is produced on (or very close to) the detection surface, allows to achieve much higher optical efficiency than that of conventional camera-based imaging systems. The performance of the biosensor was also compared with conventional benchtop instrumentation in terms of LOD and dynamic range, confirming its suitability for low-cost multiplex bioluminescence on-site applications.
Different biosensors were developed. The first detects androgenic compounds using yeast cells carrying a green-emitting P. pyralis luciferase regulated by the human androgen receptor and a red mutant of the same species as internal vitality control. The second biosensor detects two classes of compounds (androgens and estrogens) using yeast strains engineered to express green-or red-emitting mutant firefly luciferases in response to androgens or estrogens, respectively. The third biosensor detects lactose analogue isopropyl β-d-1-thiogalactopyranoside using two E. coli strains. One strain exploits the lac operon as recognition element for the

expression of P. pyralis luciferase. The other strain serves as a vitality control expressing Gaussia princeps luciferase, which requires a different luciferin substrate. A biosensors for heavy metals detection in environmental samples has been developed. The immobilized cells were stable for up to 1 month. The analytes could be detected at nanomolar levels with good precision and accuracy when the specific signal was corrected using the internal vitality control. This portable device can be used for on-site multiplexed bioassays for different compound classes and in combination with other chemilumunescent based device to set up companion diagnostics for personalized medicine [3].

[1] A.Roda, B.Roda, L. Cevenini, E.Michelini, L.Mezzanotte, P.Reschiglian, K.Hakkila, M.Virta, *Anal Bioanal Chem.,* 401, **2011**, 201

[2] A.Roda, L.Cevenini, E.Michelini, B.R. Branchini, *Biosens Bioelectron.,* 26, **2011**, 3647

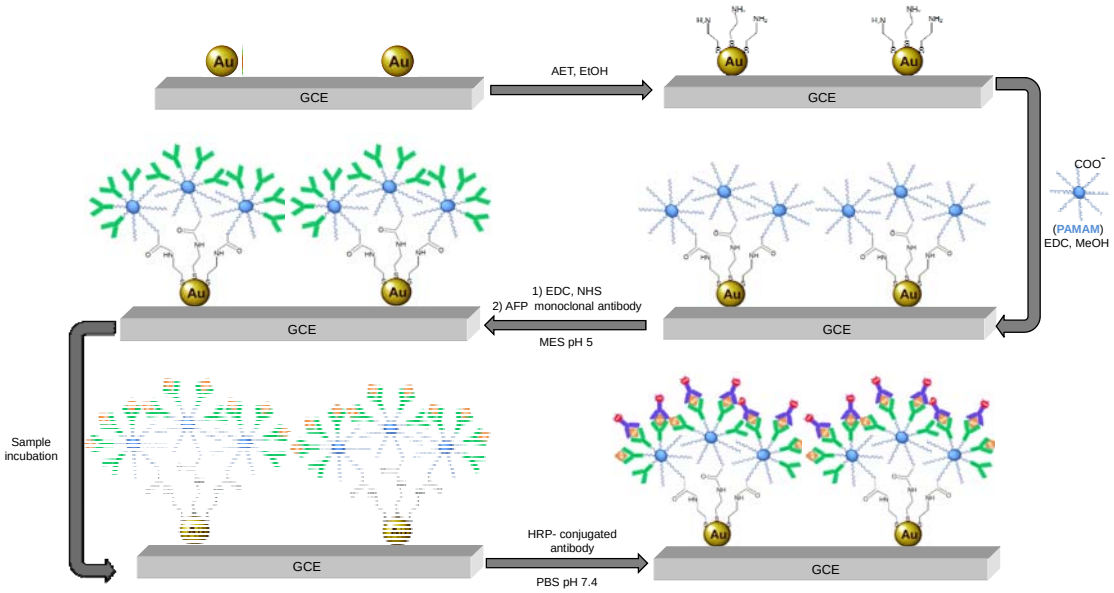
[3] A.Roda, M.Mirasoli, L.S.Dolci, A.Buragina, F.Bonvicini , P.Simoni, M.Guardigli *Anal Chem.,* 83, **2011**, 3178

ANA-OR-44 Use of polyamidoamine dendrimers anchored on nanogold for development of amperometric immunosensors based on non-competitive and competitive ELISA.

Marco Giannetto, Laura Mori, Giovanni Mori, Maria Careri, Alessandro Mangia

Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Università di Parma. Parco Area delle Scienze 17/A, 43124 Parma, Italy.
marco.giannetto@unipr.it

New amperometric immunosensors based on nanobiocomposite substrate and with response enhanced by polyamidoaminic (PAMAM) dendrimers were developed and characterized. The nanostructurated substrate obtained by electrochemical deposition of 100 nm-sized gold nanoparticles on glassy carbon electrodes (GCE) was functionalized by deposition of a self assembled monolayer of 2-aminoethanethiol (AET), used as linker for the subsequent immobilization of polyamidoaminic dendrimers (PAMAM G.1.5). This immobilization procedure was suited for the covalent linking of capture antibodies for direct non-competitive assays aimed to determination of proteins of biological interest, such as alpha-fetoprotein[1**].**



The same methodology was also investigated for the linking of haptens in order to develop competitive immunosensors for determination of small molecules of forensic or environmental interest. Studies focused on the application of PAMAM dendrimers for realization of piezoelectric immunosensors were also undertaken.

[1] M. Giannetto, L. Mori, G. Mori, M. Careri, A. Mangia, *Sens. Actuators B: Chem.* (2011), doi:10.1016/j.snb.2011.06.070

ANA-OR-45 DNA BASED MOLECULAR SWITCHES FOR THE DETECTION OF ANTIBODIES

Francesco Ricci[1], Alexis Vallee-Belisle[2], Kevin W. Plaxco[2], Giuseppe Palleschi[1]

[1]*Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma, Tor Vergata, Via della Ricerca Scientifica, 00133, Rome, Italy. Email: giuseppe.palleschi@uniroma2.it*
[2]*Department of Chemistry and Biochemistry, University of California, Santa Barbara, Santa Barbara, CA 93106 USA*

Here we report the development of a versatile DNA-based switch which enables the single-step quantitative detection of antibodies through direct optical or electrochemical outputs triggered by binding-induced structural changes. We did so by designing a DNA-based nanoswitch that is triggered by binding to two distinct sites on a single target macromolecule. By coupling this bidentate nanoswitch to optical and electrochemical outputs, we achieve the rapid (seconds/minutes), quantitative detection of sub-nanomolar concentrations of Ab raised against many antigens (*e.g.*, small molecules, peptides etc) even in highly complex samples, such as whole blood. Antibody beacons could be easily implemented in inexpensive, easy to use, electronic hand-held devices, suggesting that they may be particularly well suited for point-of-care applications. Given these attributes, we believe that Ab-beacons will enable important advances in diagnostic and Point-of-Care applications.

ANA-PO-01 Elettrodeposizione "On-Line" di Ossidi di Cobalto (III, IV) in Condizioni Alcaline per la Determinazione Amperometrica di Idrazine.

Innocenzo G. Casella[a], Michela Contursi[a].
[a]Dipartimento di Chimica dell'Università della Basilicat, Via dell'Ateneo Lucano, 10
85100 Potenza, Italy.
Innocenzo.casella@unibas.it.

Alcuni ossidi del Co(III,IV) presentano importanti attività di elettrocatalisi verso l'ossidazione di molecole organiche o la formazione/evoluzione di O_2 in condizioni neutre e/o alcaline [1,2]. L'utilizzo di sensori amperometrici a base di ossidi metallici, particolarmente in condizione di flusso (FIA, HPLC) e di elettrolisi massive prolungate, porta ad una graduale erosione della superficie elettrodica con conseguente decadimento dell'attività elettrocatalitica del sensore stesso. Un modo per affrontare questa problematica, ovvero preservare una adeguata attività catalitica, consiste nell' introdurre in continuo nel sistema di analisi, ovvero in condizioni "on-line", uno specifico mediatore redox atto ad esplicare la desiderata attività catalitica. Così la superficie elettrodica, sottoposta ad una inevitabile erosione meccanica (o di passivazione chimica) può essere rigenerata attraverso un processo di continua elettrodeposizione di nuove unità catalitiche.

La presente comunicazione riguarda una ipotesi di modificazione superficiale di elettrodi tradizionali di carbone vetroso con ossidi di Co(III, IV) in condizioni alcaline (0.1 M – 1 M NaOH), tramite l'applicazione di potenziali anodici (i.e., 0.2 V – 0.6 V vs. SCE). In tali condizioni amperometriche, il mediatore Co(III, IV), come è noto dalla letteratura, esplica la sua massima attività catalitica verso l'ossidazione di alcune importanti classi di molecole organiche [3-5] e pertanto la definizione di una procedura di elettrodeposizione di un film di Co (III,IV) in condizioni anodiche ed a pH alcalini, rappresenta un passaggio importante nella progettazione di sistemi amperometrici di analisi robusti e riproducibili. Pertanto sarà studiato un sensore amperometrico per l'analisi dell'idrazina e suoi derivati utilizzando un elettrodo di grafite e soluzioni alcaline contenenti Co-gluconato (CoL_2^{2-}) come specie modificante "on-line" dello stesso.

[1] E.B. Castro, C.A. Gervasi, Int. J. Hydr. Ener., 25, 2000, **1163**.
[2] M.E.G. Lyons, M.P. Brandon, J. Electroanal. Chem., 641, **2010**, 119.
[3] G. Cepria, J.R. Castillo, J. Appl. Electrochem., 28, **1998**, 65.
[4] I.G. Casella, M. Contursi, Anal. Bioanal. Chem., 376, **2003**, 673.
[5] A.J. Bard, M. Stratman (Ed.), Encyclopedia of Electrochemistry. Modified Electrodes. Eds. M. Fujihira, I. Rubinstein, J.F. Rusling. Vol. 10. Wiley-VCH, Weinheim, **2006.**

ANA-PO-02 Copper nanoparticles/poly-3-methylthiophene: preparation, characterization and application in glucose sensing in a flow injection system

E. Mazzotta[1], M.R. Guascito[1], C. Malitesta[1], T. Siciliano[2], M. Siciliano[2]

[a] Laboratorio di Chimica Analitica, [b] Laboratorio di Sensori Chimici
Dipartimento di Scienza dei Materiali, Università del Salento, via Monteroni 73100 Lecce, Italy
elisabetta.mazzotta@unisalento.it

The use of nanoparticles in electroanalysis is a continually expanding area of research as shown by the wealth of available research papers about the synthesis, characterization and application of nanoparticles [1]. This is due to the unique properties of nanostructured materials (e.g. enhanced mass transport, high surface area, improved signal-to-noise ratio) making their use very advantageous in many electroanalytical techniques. The advances of nanotechnology have opened up interesting research opportunities on nanocomposite fabricated not only with different nanostructured materials, but also with various conducting polymers [2]. Such composite materials have an advantage to possess properties of the individual ones with a synergistic effect [2]. In particular, the design of composite materials consisting of a mixture of organic and inorganic phases in the nanometer range has flourished in the last few years. Different strategies for fabrication of nanocomposites have been reported in literature, among which the entrapment of metal nanoparticles in conducting polymers [see: e.g. 3-5] revealed to be a simple and effective approach producing nanostructured materials with remarkable catalytic properties [6-8].

In the present work a simple non-enzymatic sensor for glucose detection has been fabricated being based on a hybrid film of electrosynthesized poly-3-methylthiophene modified by copper nanoparticles (P-3MT/CuNPs). The deposition of copper was achieved by applying a potential pulse program [9] both on Pt and on screen-printed electrodes (SPEs). The microscopic characterization of the film was performed by scanning electron microscopy/energy dispersive X-ray analysis (SEM) and showed a correlation between the pulse width and the amount and size of the deposited particles. The nanocomposite P-3MT/CuNPs was analyzed also by X-ray photoelectron spectroscopy (XPS). The electrocatalytic properties of P-3MT/CuNPs towards glucose oxidation were investigated and the composite film deposited on SPEs was used for glucose detection in a flow-injection analysis system. The effect of the applied potential as well as of the flow rate of carrier stream was evaluated: under the selected experimental conditions, the sensor revealed a satisfactory response in terms of detection limit, linear range and repeatability.

[1] F.W. Campbell, R.G. Compton, *Analytical and Bioanalytical Chemistry, 396,* **2010**, 241.
[2] Y. Xiao, C.M. Li, *Electroanalysis, 20,* **2008**, 648.
[3] P. Xu, X. Han, C. Wang, B. Zhang, X. Wang, H.-L. Wang, *Macromolecular Rapid Communications, 29,* **2008**, 1392.
[4] S. Vaddiraju, K.Seneca, K.K. Gleason, *Advanced Functional Materials, 18,* **2008**, 1929.
[5] L. Li, G. Yan, J. Wu, X. Yu, Q. Guo, *Journal of Colloid and Interface Science, 326,* **2008**, 72.
[6] J. Li, X.-Q. Lin, *Analytica Chimica Acta, 596,* **2007**, 222.
[7] A. Balamurugan, K.-C. Ho, S.-M. Chen, *Synthetic Metals, 159,* **2009**, 2544.
[8] H.-H. Shih, D. Williams, N.H. Mack, H.-L. Wang, *Macromolecules, 42,* **2009**, 14.
[9] M.R. Guascito, P. Boffi, C. Malitesta, L. Sabbatini, P.G. Zambonin, *Materials Chemistry and Physics, 44,* **1996**, 17.

ANA-PO-03 Study of the deposition of amine terminal groups on Au surfaces for the development of amperometric genosensors

Chiara Zanardi,[a] Fabio Terzi,[a] Clara Baldoli,[b] Emanuela Licandro,[c] Patrizia R. Mussini,[d] Claudio Fontanesi,[a] Renato Seeber[a]

[a]Dipartimento di Chimica dell'Università degli Studi Modena e Reggio E., Via G. Campi 183, 41125, Modena, Italy
[b]Istituto di Scienze e Tecnologie Molecolari del CNR, via C. Golgi 19, 20133, Milano, Italy
[c]Dipartimento di Chimica Organica ed Industriale, Università degli Studi di Milano, via Venezian 21, 20133, Milano, Italy
[d]Dipartimento di Chimica Fisica ed Elettrochimica, Università degli Studi di Milano, via Golgi 19, 20133 Milano
renato.seeber@unimore.it

The development of efficient enzyme biosensors, immunosensors, and genosensors is attracting the interest of scientific community, due to the need of fast responses in such an important field as that of human health. With this respect, electrochemical biosensors can also satisfy the increasing demand of low cost and easy-to-use devices.

The stable anchoring of recognition elements on the electrode surface has been generally achieved by functionalising the bio-molecule with thiol terminal groups, thanks to their high affinity with Au substrates. In the present communication we show the capability of amino terminal groups to stably interact with Au surfaces. To this aim, the deposition of hexylamine on flat Au surfaces has been studied through spectroscopic and electrochemical investigations. In particular, photoemission, X-ray absorption and vibrational spectroscopies allowed us to clarify the mechanism of the amine interaction with Au substrates, which constitutes quite a debated aspect. The stability of the adsorbed molecules on the surface and the reproducibility of the surface functionalisation have been checked through the deposition of amino derivatives bearing a ferrocene electroactive moiety.

These investigations have been exploited in the development of amperometric genosensors based on peptide nucleic acids (PNA) as recognition elements: PNA molecules functionalised with a lysine terminal group have been deposited on Au substrates and the occurrence of DNA hybridisation has been detected electrochemically. Finally, the advantages of the use of Au nanostructured surfaces, replacing the most widely used smooth ones, have been ascertained and discussed.

ANA-PO-04 Dielectric properties of lipid layers integrated in OTFT

S. Cotrone[1], M.Y. Mulla[1], M. Ambrico[2], T. Ligonzo[3], M. Magliulo[1], G. Palazzo[1], A. Mallardi[4], M. D. Angione[1], N. Cioffi[1], L. Torsi[1]

[1] Università di Bari, Dipt. di Chimica, Via Orabona 4, 70126 Bari, Italy
[2]CNR-IMIP, Sezione Territoriale di Bari, Via Orabona 4, 70126 Bari, Italy
[3] Università di Bari, Dipt. Interateneo di Fisica,Via Amendola, 172, 70126 Bari, Italy
[4] Istituto per i Processi Chimico-Fisici (IPCF), CNR – Bari (Italy)
Email: yusuf.mulla@uniba.it

Recent advancements in the electronic devices based on organic materials have opened up novel applications of Organic Thin Film Transistors (OTFT) based biosensors such as, chemical sensing and label free detection [1]. The use of cell-membrane mimics such as liposome and lipid bilayers has recently attracted great attention in the OTFT sensors and several immobilization methods on a solid substrates have been reported [2-3]. Supported membranes show an intrinsically low bioactivity, making them interesting as an interface between the non-biological material on the surface of OTFT and biologically active fluids. The dielectric properties of such supported membranes are critical for the electrical performance of OTFT based sensors, because majority of the biomaterials possess insulating properties [4].
Investigation of the dielectric properties of supported lipid bilayer membrane (sBLM) has been done using impedance spectroscopy in two steps. In the first one, sBLM has been characterized directly on a metal support and in the second an organic semiconductor was used to improve the quality of the supported lipid bilayer. The sBLMs structure in the two systems is explained in terms of equivalent circuits composed of resistors and capacitors. Information on the presence of lipid bilayer inhomogeneities are gathered from the electrical parameters determined by fitting the frequency-dependent impedance of the equivalent circuits to the measured data. Furthermore, X-ray photoelectron spectroscopy was employed for the characterization of the active layers, in terms of surfaces and interfaces quality, e.g surface elemental composition etc.

[1] L. Torsi, G.M. Farinola, F. Marinelli, M.C. Tanese, O.H. Omar, L. Valli, F. Babudri, F. Palmisano, P.G. Zambonin, F. Naso, *Nat. Mater*. 7, 2008, 412.
[2] H. Lee, H. Jung, K. Fujikawa, J. Park, J. Kim, T. Yukimasa, H. sugihara, T. Kawai, *Biosens and Bioelectron*., 21, 2005,833.
[3] I. Pfeiffer and F. Höök, *J. Am. Chem. Soc.*, 126 (33), 2004,10224.
[4] C. Bettinger and Z. Bao, *Polym Int*, 59, 2010,563.

ANA-PO-05 Reliability testing in OFET Biosensors

M.D.Angione[a], L.M. Dumitru[a] , M. Magliulo[a], S. Cotrone[a] , A. Mallardi[b], N. Cioffi[a], G. Palazzo[a] and L.Torsi[a]

[a] University of Bari "Aldo Moro" – Bari (Italy)
[b] CNR-IMIP, Sezione Territoriale di Bari, Via Orabona 4, I-70126 Bari, Italy
E-mail address: liviu.dumitru@uniba.it

A lot of efforts have been invested in more recent years to improve the specificity and selectivity for the detection of chemical and biological species. A lot of analytical well established methods have been used in the past for the detection of chemical and biological analytes, such as mass-spectrometry, enzyme linked immunosorbent (ELISA) assays, gas and liquid chromatograph. These methods although high throughput and reliable, require extensive sample treatment involving incubation steps or amplifications.

The advantage of using Organic Field Effect Transistors as transducing tools for biosensor applications, resides in the chance of avoiding, or at least minimizing the sample pre-treating, fabrication costs, and to open up new perspectives in the development of portable and disposable device for a large area of applications.

Recently OFET biosensors have shown, in fact, the potential to offer [1] very high performance level while organic electronics allow to fabricate sensing circuits, also in an array configuration [2], on flexible, paper substrates by low cost printing procedures. The coupling of the OFET device and the biological recognition element can be actuated by assembling supramolecular structures embedding biomolecules, and depositing them directly over the OFET active layer.

A comparison of different deposition procedures based on the use of phospholipids bilayers will be presented, providing also morphological and structural investigations. Preliminary reliability data for this novel bio-OFET will be also presented.

The incorporation of biomaterials into an organic based electronic device has the potential for unique applications in medicine and point-of-care diagnostics. Future efforts will need to be focused on the development of new hybrid biomaterials integrated into electronic devices capable of being processed on flexible substrates.

[1] L. Torsi, et al., *Nat. Mater.*, 7, **2008**, 412.
[2] T. Someya et al., PNAS 101, **2004**, 9966.

ANA-PO-06 Spectroscopic characterization of Te-based micro- and nanostructured materials for sensing applications

M.R. Guascito[a], R.A. Picca[a], D. Chirizzi[a], E. Mazzotta[a], T. Siciliano[b], M. Siciliano[b], C. Malitesta[a], A. Tepore[b]

[a]Laboratorio di Chimica Analitica, Dipartimento di Scienza dei Materiali, Università del Salento, Via Monteroni, 73100 Lecce, Italy
[b]Laboratorio di Crescita di Cristalli, Dipartimento di Scienza dei Materiali, Università del Salento, Via Monteroni, 73100 Lecce, Italy
rosanna.picca@unisalento.it

Micro- and nanomaterials have emerged as advanced tools in several research and technology applications because of the possibility of tuning material properties reducing their size [1]. Especially nanoparticles with an anisotropic morphology (e.g. tubes and wires) are advantageous because they possess a high surface area and multiple contacts (borders, inner and/or outer surfaces) that in principle can be functionalized in several ways [1]. In particular, Tellurium microtubes have been successfully proposed in gas and liquid sensors [2, 3]. Recently, Te(IV) oxide nanowires (NWs) have been synthesized by thermal evaporation of Te(0) in an oxygen atmosphere [4] and their direct growth onto Pt substrates to develop novel electrochemical sensors is proposed in the present contribution. In particular, the intimate contact between NWs and Pt and their potential modification upon electrochemical treatments have not been addressed. Such issue regarding surfaces and interfaces TeO_2 NWs/Pt may be of particular relevance in terms of sensing applications. Moreover, only few studies have addressed such topic (mainly on elemental Te [5] and tellurides [6]) by X-ray Photoelectron Spectroscopy (XPS). In this communication we report preliminary results on XPS study in relation to electrochemical and X-Ray Diffraction (XRD) analyses on TeO_2 NWs grown on Pt electrodes. A comparison with results on Te(0) microtubes will be also presented.

[1] G.R. Patzke, F. Krumeich, R. Nesper, *Angew. Chem. Int. Ed.* **2002**, *41*, 2446.
[2] T. Siciliano, E. Filippo, A. Genga, G. Micocci, M. Siciliano, A. Tepore, *Sens. Actuators, B* **2009**, *142*, 185.
[3] M.R. Guascito, D. Chirizzi, C. Malitesta, E. Mazzotta, M. Siciliano, T. Siciliano, A. Tepore, A. Turco, *Biosens. Bioelectron.* **2011**, *26*, 3562.
[4] T. Siciliano, A. Tepore, G. Micocci, A. Genga, M. Siciliano, E. Filippo, *Sens. Actuators, B* **2009**, *138*, 207.
[5] W.P. Zhou, L.A. Kibler, D.M. Kolb, *Electrochim. Acta* **2002**, *47*, 4501.
[6] H.K. Edwards, P.A. Salyer, M.J. Roe, G.S. Walker, P.D. Brown, D.H. Gregory, *Cryst. Growth Des.* **2005**, *5*, 1633.

ANA-PO-07 INNOVATIVE ELECTRODES TO CONTROL TRACE METAL IONIZATION USED TO TREAT PATHOGENS IN WATER DISTRIBUTION SYSTEMS

Diego Voccia, Serena Laschi, Ilaria Palchetti, Marco Mascini, Giovanna Marrazza

Dipartimento di Chimica "Ugo Schiff", Università di Firenze, Via della Lastruccia 3, 50019 Sesto Fiorentino (FI)
giovanna.marrazza@unifi.it

The control of hazardous pathogens in water distribution systems, is a priority for health authorities world wide. An estimated 8,000 to 18,000 people get Legionnaires' disease in the United States each year. Hospitals, hotels, old people's homes, prisons and ships are high risk environments due to the nature of the water distribution system. Treatment is essential, and one of the most effective methods is copper-silver ionization. The positively charged copper and silver ions thus released, form electrostatic bonds with negatively charged sites on bacterial cell walls; this leads to cell lysis and cell death. Importantly, some authors have demonstrated that these ions are able to penetrate the biofilms in which other bacteria, algae, protozoans, and fungi cohabit with Legionella species in water pipes. The amount of copper and silver must remain within a certain range for efficiency, and at the same time remain well below the WHO and other guidelines. High oral intake of copper and silver can result in liver failure and argyria (blue-bluish grey discoloration of the skin) for copper and silver respectively. Recommended values for copper are between 0.3 and 0.5 mg l^{-1} and, for silver, between 0.03 and 0.05 mg l^{-1}.
The specific aim of this work was to study the electrochemical behaviour of screen-printed graphite electrodes in the determination of silver and copper, with the final purpose of development and construction of mercury-free electrodes to be used in the determination of silver and copper concentrations in water samples by anodic stripping voltammetry.
Particular attention was focused on the chemistry of complex formation in solution optimizing pH and reagents concentration to obtain the better reproducibility, dynamic range and selectivity.

ANA-PO-08 A proteomics approach to study as DNA affects the composition of lipoplex protein corona

Anna Laura Capriotti,[a] Giulio Caracciolo,[b] Chiara Cavaliere,[a] Patrizia Foglia,[a] Daniela Pozzi,[b] Roberto Samperi,[a] Aldo Laganà[a]

[a]Dipartimento di Chimica della Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185, Rome, Italy,
[b] Dipartimento di Medicina Molecolare della Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185, Rome, Italy
annalaura.capriotti@uniroma1.it

The in vivo organ distribution of intravenously administered drug carriers strongly depends on plasma proteins adsorbed onto their surface, furthermore, a precise knowledge of the structure and morphology of drug carriers is relevant to understand their role as gene delivery [1]. In the present study, we investigated and compared the binding of human plasma proteins onto CLs and onto their relative DNA lipoplexes. A shotgun proteomics approach based on high performance liquid chromatography coupled to high resolution mass spectrometry was employed for an efficient identification of proteins adsorbed onto liposome and lipoplex surface. The distinct pattern of proteins adsorbed helps to better understand the DNA compaction process. The experimental evidence leads us to hypothesize that negatively charged DNA is adsorbed onto lipoplex surface and can interact with basic plasma proteins, in agreement with the existence of cluster-like aggregates made of multilamellar DNA/lipid domains coexisting with other multilamellar lipoplexes or, alternatively, with DNA-coated vesicles. Proteomics experiments showed that the lipoplex corona is rich of biologically relevant proteins such as fibronectin, histones and complement proteins. Our results provide novel insights to understand how lipoplexes activate the immune system and why they are rapidly cleared from the blood stream. The differences in the protein adsorption data detected in the presented experiments could be the basis for the establishment of a correlation between protein adsorption pattern and in vivo fate of intravenously administered nanoparticles and will require some consideration in the future.

[1] D. Walczyk, F. Baldelli Bombelli, M.P. Monopoli, I. Lynch, K.A. Dawson, *J. Am. Chem. Soc*., *132*, **2010**, 5761.

ANA-PO-09 An analytical strategy for studying proteins differentially adsorbed onto surface of three liposome formulations

Giuseppe Caruso,[a] Giulio Caracciolo,[b] Carlo Crescenzi,[c] Maria Anna Euterpio,[c] Daniela Pozzi,[b] Roberto Samperi,[a] Aldo Laganà[a]

[a] Dipartimento di Chimica della Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185, Rome, Italy
[b] Dipartimento di Medicina Molecolare della Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185, Rome, Italy
[c] Dipartimento di Scienze Farmaceutiche e Biomediche Università di Salerno, Via ponte don Melillo, 84084 Fisciano (SA), Italy
giuseppe.caruso@uniroma1.it

The knowledge about the interaction between plasma proteins and nanocarriers employed for in vivo delivery is fundamental to understand their biodistribution [1]. Protein adsorption onto nanoparticle surface (protein corona) is strongly affected by vector surface characteristics. In general, the primary interaction is thought to be electrostatic, thus surface charge of carrier is supposed to play a central role in protein adsorption. Because protein corona composition can be critical in modifying the interactive surface that is recognized by cells, characterizing its formation onto lipid particles may serve as a fundamental predictive model for the in vivo efficiency of a lipidic vector.

In the present work, protein coronas adsorbed onto three differently charged cationic liposome formulations were compared by a shotgun proteomic approach based on nano-liquid chromatography coupled to high resolution mass spectrometry. About 130 proteins were identified in each corona, with only small differences between the different cationic liposome formulations. In particular, surface charge did not show to drive a preferential protein absorption. Therefore, to better understand the interactions between the single liposome formulation and plasma proteins, a label free quantitative analysis was performed by the aid of the software Scaffold. The results allowed to point out the differences not found out from the simple qualitative analysis.

This study could be useful for the future controlled design of colloidal drug carriers and possibly in the controlled creation of biocompatible surfaces of other devices that come into contact with proteins into body fluids.

[1] T. Cedervall, I. Lynch, M. Foy, T. Berggård, S.C. Donnely, G. Cagney, S. Linse, and K.A. Dawson, *Angew. Chem. Int. Ed.*, *119*, **2007**, 5856.

ANA-PO-10 Near infrared spectroscopy and class modelling techniques for the verification of authenticity of Taggiasca table olives

S. Lanteri[a*], M.C. Casolino[a], M. Casale[a], P. Oliveri[a], L. Bagnasco[b], M.E. Cosulich[b,c], F. Mare[d], L. Medini[d]

[a] University of Genoa, Department of Pharmaceutical and Food Chemistry and Technologies, Via Brigata Salerno 13, I-16147 Genoa, Italy
[b]University of Genoa, Department of Chemistry and Industrial Chemistry, Via Dodecaneso, 31 – I-16146 Genoa, Italy
[c]University of Pavia, Department of Biochemistry, Via Taramelli, 3B – I-17100 Pavia, Italy
[d]Azienda Speciale per la formazione professionale e la promozione tecnologica e commerciale, Laboratorio Chimico Merceologico, Regione Rollo, 98 – I-17031 Albenga (SV), Italy
Email: silvia@dictfa.unige.it

The Mediterranean coastal areas are characterised by a mild, warm climate – an ideal habitat for growing of *Olea europaea* L. tree. *Taggiasca* is a typical Ligurian olive cultivar, whose cultivation is very important for the local economy and the preservation of the regional landscape. A part of *Taggiasca* crop is used to prepare table olives, which can be easily adulterated with olives having similar morphological features but a lower commercial value. The potential of near-infrared (NIR) spectroscopy and multivariate analysis to characterise the *Taggiasca* olives has been evaluated in the present study.

A considerable number of samples of table olive of *Taggiasca* cultivar and of cultivars *Leccino* and *Coquillo* were collected and analysed. The traceability and representativeness of sampling was assured.

Olives were washed with water, dried and stoned, then the pulp was ground and submitted to spectroscopic analysis in the reflectance mode. The measurements were replicated in two different laboratories and with two different spectrophotometers: a FT-NIR Buchi based on a polarisation interferometer (NIRFlex N-500) and a FT-NIR Thermo Scientific (AntarisII FT-NIR Analyzer). This allowed to evaluate the reproducibility of the method proposed. Principal component analysis (PCA) was applied to visualise the multivariate data distribution. Then, two class modelling techniques (soft independent modelling of class analogy - SIMCA, unequal class models - UNEQ) were tested to characterise *Taggiasca* olives.

For both of the laboratories, the PCA score plots showed a satisfactory distinction between the three cultivars. This result was confirmed by the supervised techniques (SIMCA and UNEQ), which allowed to build efficient class-models for the verification of authenticity of olives.

The present study demonstrated the potential of NIR spectroscopy coupled with multivariate analysis as a rapid and low-cost tool to characterise olives.

ANA-PO-11 Multivariate methods for experimental-data analysis applied to identification of bacteria by means of Py/GC-MS

Dora Melucci, Simona Montalbani, Simona Raffo

Dipartimento di Chimica Ciamician, Università di Bologna, *dora.melucci@unibo.it*

There is the need to identify bacteria by means of reliable but rapid analytical methodologies: the already assessed current analytical methodologies require laborious preparative steps; moreover, they are often very expensive. In order to solve these problems, a more rapid and less expensive analytical technique based on Py/GC-MS has been here developed.

Analytical pyrolysis (Py) allows the characterization of a material by controlled thermal chemical degradation in inert atmosphere; degradation products are characteristic of the original sample and can be characterized using a GC-MS system [1]. When Py/GC-MS is applied to bacteria, trivial visual examination of *pyrograms* easily allows discrimination between different *genera* of bacteria. But to obtain discrimination in terms of *species* chemometric data analysis is necessary.

Among chemometric methods for *data exploration*, *Principal Components Analysis* (*PCA*) is extensively used in Analytical Chemistry. As for *classification procedures*, Soft Independent Modeling of Class Analogy (SIMCA) is particularly suitable for analytical problems, since it works even when the number of samples is very low with respect to the number of variables characterizing samples.

The purpose of the present work was to develop a rapid Py/GC-MS methodology for the analysis and identification of bacteria in terms of *genus* and *species*. Methylated fatty acids (FAMEs) were chosen as biomarkers of bacteria in the pyrolysates. *In situ* Thermal Hydrolysis and Methylation (THM) was applied. Pyrographic peak areas, and relevant normalized or relative values, were chosen as variables for chemometric data processing by PCA and SIMCA.

Satisfactory results were obtained in classifying bacteria in terms of *genus* and *species*.

[1] D. Melucci, G. Chiavari, S. Montalbani, S. Prati, *J. Therm. Anal. Calorim.* **104** (2011) 415-421

ANA-PO-12 Experimental design optimization of a solid-phase microextraction coupled with gas chromatography-tandem mass spectrometry methods for the determination of carbamate pesticides in water

Marcello Monteleone, Attilio Naccarato, Giovanni Sindona, Antonio Tagarelli

Dipartimento di Chimica, Università della Calabria, via P. Bucci Cubo 12/C 87036 Arcavacata di Rende (CS)
attilio.naccarato@unical.it

Carbamate pesticides are a wide family of pesticides whose structures ($R_1OCONR_2R_3$) are derived from carbamic acid by the introduction of different substituent [1]. They are class of synthetic organic pesticides annually used on a large scale worldwide as insecticides, herbicides, fungicides, nematocides, acaricides and molluscicides. Most of the carbamates have high melting points and low vapor pressure and are often present in aqueous environments because of their high solubility in water [2]. This class of pesticides are toxicity to the central nervous system and they are suspected carcinogens and mutagens. The European Union has set a maximum admissible concentration of 0.5 μg/L for the sum of all pesticides and 0.1 μg/L for an individual compound in drinking water [3]. Due to their thermal instability their analytical determination is usually carried out by HPLC techiques [4]. The purpose of this work was the development of an solid-phase microextraction coupled with gas chromatography-tandem mass spectrometry (SPME-GC-MS/MS) analytical method, for the determination of six carbamate pesticides in water (aldicarb, propoxure, carbofuran, carbaryl, methiocarb and pirimicarb). Experimental design was used in order to select the optimal SPME and gas chromatographic experimental conditions which allow the controlling of thermal degradation of analytes and their successful extraction avoiding on-fiber memory effects. Carbamates quantification was carried out using two internal standards (carbaryl-d7 and trimethacarb). Triple quadrupole tandem mass spectrometry was applied to decrease the limit of detection (LOD) and the limit of quantification (LOQ) for the analytes using multiple reaction monitoring mode (MRM). The method developed shows accuracy and precision levels between 70-108 % and LOQ values between 0.06-2.9 ng/L.

[1] M.J. Climent and M.A. Miranda *J.Chromatogr. A*,738, **1996**, 225.
[2] Y. Gou, R. Eisert and J. Pawliszyn *J.Chromatogr. A*,873, **2000**, 137.
[3]Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption, Official Journal L 330, European Union, Brussels, 5 December 1998, p. 32.
[4]M.J. Santos Delgado, S.R. Barroso, G. Toledano Fernandez-Tostado, L.M. Polo-Diez *J.Chromatogr. A*,921, **2001**, 287

ANA-PO-13 FAST ANALYSIS OF SOME PHENOLICS BY HPLC-DAD AND CHEMOMETRIC RESOLUTION TECHNIQUES

Riccardo Nescatelli, Federico Marini, Remo Bucci, Antonio L. Magrì, Andrea D. Magrì

Dipartimento di Chimica, Università di Roma "La Sapienza", P.le Aldo Moro 5, 00185 Roma, Italy
riccardo.nescatelli@uniroma1.it

In recent years, the prevention of cardiovascular diseases and cancer has been intensively focused on the beneficial effects of olive oil and, in particular, of its phenolic pool. These species are powerful inhibitors of the formation of atherosclerotic plaques and reactive oxygen species, cause of "fat-related" cancers.
It seems clear that the phenolic composition represents a specific index of nutritional quality of the product and how important it is to develop rapid, economic and simple analytical methods for its determination in olive oils.
To this end, since chemometrics allows the mathematical a posteriori resolution of overlapping peaks, it is possibile to operate with experimental conditions even leading to a not perfect chromatographic separation, with a corresponding saving of money and time and reduction in the amount of solvents used. Accordingly, in this communication, the possibility of operating a rapid HPLC-DAD analysis of a wide range of polyphenols, using a fast gradient involving two solvents only ($MeOH/H_2O$) and chemometric multivariate and multiway techniques, will be discussed. In particular, two approaches were tested for the resolution of peak clusters: the use of Multivariate Curve Resolution (MCR [1]) after unfolding of the experimental data cube and the application of PARAFAC2 [2] directly on the three way array. The results obtained by both methods have been validated by comparison with a complete chromatographic resolution of the co-eluted peaks. In particular, it was shown that both chemometric approaches led to good results but MCR performed better on the investigated data set. A possible explanation of this outcome could be found in the fact that, as the spectral profiles of the analytes are very similar, further constraints are needed to separate the contribution of the individual components, for instance unimodality of the peaks, and these constraints can't be applied in PARAFAC2 due to algorithmic reasons.

[1] R. Tauler, A. De Juan, Crit. Rev. Anal. Chem., 36 (2006) 163-176.
[2] H.A.L. Kiers, J.M.F. ten Berge, R. Bro, J. Chemometrics 13 (1999) 13, 275-294.

ANA-PO-14 Spectrophotometric and DFT characterization of uranyl carboxylate complexes in aqueous solution

Silvia Berto[a)], Pier Giuseppe Daniele [a)], Eliano Diana[b)], Enrico Prenesti[a)],

[a)] Dipartimento di Chimica Analitica dell'Università, Via Pietro Giuria 5, 10125 Turin, Italy
[b)] Dipartimento di Chimica IFM dell'Università, Via Pietro Giuria 7, 10125 Turin, Italy
e-mail address of the presenting author: piergiuseppe.daniele@unito.it

This investigation is an advance in the understanding of uranyl chemistry in water solution. Our previous works [1-3] were addressed to the speciation of uranyl with carboxylic acids by potentiometry and UV-visible absorption spectrophotometry. In this session we propose an enhancement on structural description of complexes by means of a deep analysis of spectrophotometric absorption data and by means of DFT (Density Functional Theory) characterization. The spectrophotometric study of metal - ligand systems can provide information about the structure of complexes, especially if it is supported by theoretical data. Moreover, the recent literature confirms that the theoretical DFT approach provides complementary information and validates the structural and solution chemical information [4]. We report the investigation on coordination compounds of uranyl ion with citric and oxydiacetic (diglycolic) acids in aqueous solution. The different binary systems were previously studied [2, 3] by potentiometric and spectroscopic techniques at t = 25 °C and I = 0.1 mol dm^{-3}. A speciation model was proposed for both metal/ligand systems from potentiometric data. Moreover, the joint elaboration of potentiometric and spectroscopic data obtained on the uranyl - ligand containing solutions allowed us to calculate the individual spectra of the complex species. With the carboxylate ligands we have found that the co-ordination environment produces an increase in the molar absorptivity values and a light bathochromic shift in the position of ε_{max} [1-3]. The bathochromic shift is higher for citric acid [3] and we observed a remarkable raise of the relative intensity of the vibronic bands which appear at λ>440 nm for uranyl-oxydiacetate complexes.

Uranyl - carboxylate species assumed from potentiometric speciation, have been checked by means of a computational modelization. Differing coordination geometries have been explored, both in gas phase and in solution (assuming the polarizable continuum model). The different protonation constants of uranyl - citrate complexes have been analyzed and electronic and vibronic spectra computed with time - dependent density functional method. Results are compared with experimental spectra.

[1] S. Berto, F. Crea, P.G. Daniele, C. De Stefano, E. Prenesti, S. Sammartano, V. Zelano, *Ann. Chim. (Rome)*, ***96***, **2006**, 399-420
[2] S. Berto, F. Crea, P.G. Daniele, C. De Stefano, E. Prenesti, S. Sammartano, *J. Sol.Chem.*, ***38***, **2009**, 1343-1356
[3] S. Berto, F. Crea, P.G. Daniele, C. De Stefano, E. Prenesti, S. Sammartano, *Radiochimica Acta*, **2011**, submitted.
[4] J. Vázquez, C. Bo, J.M. Poblet, J. de Pablo, J. Bruno, *Inorg. Chem*., ***42***, **2003**, 6136-6141

ANA-PO-15 Sequestering ability of S-donor ligands towards metal and organometal cations

Gabriella Falcone, Claudia Foti, Ottavia Giuffrè and Silvio Sammartano
Dipartimento di Chimica Inorganica, Chimica Analitica e Chimica Fisica dell'Università, Via F. Stagno d'Alcontres 31, 98166 Messina (Vill. S. Agata), Italy
cfoti@unime.it

Sulfur containing compounds play a key role in protecting biological systems against oxidative injury and heavy metal intoxication. The ability of many of these compounds to penetrate cellular membranes has made them useful chelating agents in the treatment of metal poisoning (*chelating therapy*). The use of a ligand as chelating agent requires the knowledge of both coordination models and formation constants of complexes with the metal ions to compare the strength and the characteristic of the species. Therefore, the study of the solution chemistry of metal ions in the presence of sulfur containing ligands can provide useful information regarding the nature and magnitude of the complexes so formed and represents an essential first step in the development of models to predict the metal transport and fate. In turn, this information can be used to evaluate methods of removing undesirable compounds from biological systems.

For these reasons, we planned a study on the solution chemistry of different metal (Hg^{2+}, Zn^{2+} and Pb^{2+}) and organometal (CH_3Hg^+, $(CH_3)_2Sn^{2+}$ and $(CH_3)_3Sn^+$) cations in the presence of sulfur containing ligands. In particular, a natural occurring detoxificant (glutathione, *gsh*) and some pharmaceutical chelating agents [2-mercaptopropanoic acid (*tla*), 3-mercaptopropanoic acid (*mpa*), 2-mercaptosuccinic acid (*tma*), penicillamine (*pen*) and L-cysteine (*cys*)] were selected as case studies. The thermodynamic parameters were determined by potentiometry (ISE-H^+), ^{1}H-NMR and titration calorimetry, at different ionic strength ($0.1 \leq I \leq 1$ mol·L^{-1}) and temperature ($15 \leq t \leq 45$°C). For all systems, the results showed the formation of mononuclear ML and MLH and binuclear ML_2 species, and, in some cases, the formation of other protonated MLH_2, ML_2H, ML_2H_2 and mixed hydrolytic ML(OH) species. As expected, the higher stability was found for species of Hg^{2+} and CH_3Hg^+, owing to the strong affinity between a typical soft metal, such as Hg(II), and a ligand with soft donor atoms, such as S.

Formation constants and speciation profile for each metal-S donor ligand systems, were useful to quantitatively define the sequestering ability of sulfur containing ligands towards methylmercury(II) ion. Sequestering ability of ligands towards metal cations was quantitatively evaluated by determining an empirical parameter ($pL_{0.5}$) that numerically represents the ligand concentration [-log (total ligand concentration)] necessary to sequester the 0.5 of metal ion fraction. The $pL_{0.5}$ values were evaluated for all systems, in different conditions of temperature and ionic strength.

ANA-PO-16 Influence of mixed $metal_1$ – $metal_2$ – ligand and metal – $ligand_1$ – $ligand_2$ species on the speciation of multicomponent aqueous solutions

Rosalia Maria Cigala, Francesco Crea, Concetta De Stefano, Gabriele Lando, Demetrio Milea, Silvio Sammartano

Dipartimento di Chimica Inorganica, Chimica Analitica e Chimica Fisica, Università di Messina, V.le F. Stagno d'Alcontres, 31, 98166 Messina
dmilea@unime.it

Natural waters and biological fluids, as well as wastewaters, can be considered, from a chemico-physical point of view, as multielectrolyte aqueous solutions in which a wide number of cations and ligands (including water / OH^-) are simultaneously present in different concentration ranges and ratios. This variability determines the formation of several complex species in solution, of different stability. Moreover, it is important to take into account that the most of these cations and ligands forms many polymeric species, also at low concentration. As a result, the formation of hetero-cationic or hetero-ligand mixed species is frequent in the conditions of these multicomponent aqueous solutions. Unfortunately, these mixed species are very often neglected in the formulation of various speciation models, though it has been demonstrated that they strongly affect the reliability of these models, especially in relation to the fact that the formation of mixed species is often thermodynamically favored with respect to parent ones. For all these reasons, since some years our group undertook various studies in this direction, in order to evaluate the formation and the stability of several mixed $metal_1$ – $metal_2$ – ligand and metal – $ligand_1$ – $ligand_2$ species in aqueous solution. In this contribution, some results of these investigations are reported.

[1] M. T. Beck, I. Nagypàl, *Chemistry of Complex Equilibria*, Orwood, New York, **1990**.
[2] F. Crea, D. Milea, S. Sammartano, *Talanta, 65*, **2005**, 229.

ANA-PO-17 Comparison of accelerated nonisothermal DSC and long-term isothermal measurements to evaluating kinetic data and predictive model for isothermal degradation time in two commercial acetylsalicylic acid-based tablet formulations

Mauro Tomassetti, [a] **Stefano Vecchio,** [b] **Luigi Campanella,** [a] **Tania Gatta,** [a]

[a]Dipartimento di Chimica di Sapienza Università di Roma, P.le A. Moro 5, 00185, Roma, Italy
[b]Dipartimento S.B.A.I. di Sapienza Università di Roma, Via del Castro Laurenziano 7, 00161, Roma, Italy
mauro.tomassetti@uniroma1.it

Differential scanning calorimetry was used as a rapid screening technique to study the stability of acetylsalicylic acid (ASA), pure or contained in two commercially available pharmaceutical formulations, where ASA is present at a high nominal concentration, along with some of the most commonly used excipients (starch, cellulose, saccharin).

The stability study was focused on the kinetic analysis of the thermal decomposition of ASA, which occurs in pure ASA as well in the two pharmaceutical formulations (PF1 and PF2) using two well–known multi–heating model–free kinetic methods: Kissinger and Ozawa–Flynn–Wall. The knowledge of the Arrhenius parameters related to this process (activation energy E_a, pre–exponential factor A, kinetic constant k) enable to calculate the half–life time values (extrapolated at 25 °C), calculated at fixed percentages of product degraded for pure ASA as well for both dosage forms considered. Finally, the results of long–term isothermal measurements, consistent with short–term non–isothermal (accelerating) ones, provided a reasonable predictive model to calculate the isothermal degradation times, thus demonstrating the reliability of extrapolated half–life time values obtained in this study.

Half–life values for degradation of pure ASA was found to be higher than those of ASA contained in PF1 and PF2, thus demonstrating that the presence of these excipients (even though their content in the dosage forms tested is very low in both cases) has a non negligible destabilizing effect.. Very reasonable degradation time values were obtained from accelerated stability experiments from pure ASA, PF1 and PF2, whose reliability was confirmed by long–time experiments, carried out at constant temperature (70 °C) for different time values.

ANA-PO-18 Identification and quantification of berberine and its main metabolites in human plasma by HPLC-ES-MS/MS

Carolina Colliva, Marinella Roberti, Cristina Ianni, Claudio Calvanese, Andrea Lisotti, Giuseppe Mazzella, Aldo Roda

Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126, Bologna, Italy
Department of Clinical Medicine, University of Bologna, Via Massarenti 9, 40138, Bologna, Italy
Carolina.colliva2@unibo.it

Berberine is an isoquinoline alkaloid of the protoberberine type, usually found in the roots, stems, rhizomes and bark of plants such as *Berberis, Hydrastis, Coptis,* etc. Recently, clinical trials have shown its usefulness in the treatment of hypercholesterolemia. Indeed, administration of berberine determines a decrease of the levels of total cholesterol, LDL-cholesterol and triglycerides levels by a dual mechanism of action: inhibition of the synthesis of triglycerides and up-regulation of the activity on the low-density-lipoprotein receptor (LDLR).
The extensive use of the therapeutic agents called "nutraceuticals", which do not require a conventional drug approval trial in term of pharmacokinetics, metabolism and safety, evidenced the need to monitor their blood levels to avoid undesiderable side effects (i.e., ipertension in the case of berberine). At present, only a few chromatographic methods are sufficiently sensitive for the determination of berberine and its metabolites in human plasma. In the present study berberine, its major metabolites (including berberrubine and demethyleneberberine synthetized in our laboratory) and their glucuronide conjugates were quantified in human plasma by high performance liquid chromatography/tandem mass spectrometry with electrospray ionization (HPLC-ES-MS/MS).

The chromatographic separation was performed at a flow rate of 0.3 mL/min using a Luna C18 column at a temperature of 40°C, and with a mobile phase constituted by a gradient of 10 mM formic acid (pH = 4.00):acetonitrile. The analysis time was 15 min. Detection was performed by Multiple Reaction Monitoring (MRM) operating in the positive ionization mode, by monitoring the transitions at m/z 336→320 (berberine), m/z 322→307 (berberrubine), m/z 322→307 (thalifendine), m/z 324→280 (demethyleneberberine), m/z 338→323 (jatrorrhizine), and m/z 414→220 (IS noscapine). The method was validated according to current guidelines (Guidance for Industry: Bioanalytical Method Validation, FDA, 2001) and applied to pharmacokinetic studies aimed at increasing the bioavailability of berberine after chronic oral administration. The developed method fulfil all the standard requirements of precision and accuracy with a LOQ of 0.5 ng/mL and is therefore appropriate for the quantification of berberine plasma levels after chronic feeding of berberine at a daily dose of 500 mg.

ANA-PO-19 A new methodology for the analysis of bile acid profile in human serum based on an ultrafiltration clean-up step and LC-MS/MS analysis.

Camilla Montesano[a], Manuel Sergi[b], Sabino Napoletano[a], Daniel Pizzoni[a], Dario Compagnone[b], Roberta Curini[a], Giuseppe D'Ascenzo[a], Franco Colistro[c]
[a] Department of Chemistry, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy;
[b] Department of Food Science, University of Teramo, Via Carlo Lerici 1, 64023 Mosciano S.A. (TE), Italy;
[c] Biochemistry Laboratory, Bambino Gesù Children's Hospital-Rome, Piazza S. Onofrio, 4, 00165 Rome, Italy
e-mail: camilla.montesano@uniroma1.it; msergi@unite.it

Bile acids (BAs), the major end products of cholesterol catabolism, are useful biomarkers for the diagnosis of many diseases. The pathologies related with bile acids are generally expressed in the first years of life and may lead to serious liver injury. Usually the balance between bile acid synthesis, secretion and re-absorption is tightly regulated: elevated concentrations of bile acids in peripheral circulation are evidence of disorders; similarly, low concentrations may suggest inborn errors of bile acid metabolism [1]. Measurement of total bile acids, as often performed in routine analysis, is only of limited value whereas the analysis of BAs profile in body fluids is an important tool to establish the therapy (pharmacological or surgical) [2].

Here we present a sensitive and rapid method for the analysis of the main 15 bile acids in human serum by liquid chromatography-tandem mass spectrometry. The chromatographic separation is performed using a core-shell column which provides a good separation, particularly useful because of the small structural differences of the analytes. All isomeric BAs of interest were resolved from each other in less than 10 min. Serum sample pretreatment only requires an ultrafiltration step with centrifugal filter devices. This simple procedure on the one hand allows a minimal consumption of serum sample (about 100 μl) and on the other hand is simple, rapid and easily applicable in a routine analysis getting anyhow a satisfying clean up. The calibration curves were linear for all the BAs over a range of 0.005–5 ppm. The extraction recoveries for all the analytes were greater than 80%. Intra-day and inter-day coefficients of variation were all below 15%. The method proposed has been validated according to FDA guidelines for bioanalytical methods and has been applied for the serum analysis of pediatric patients.

[1] D.W. Russel, *Annu. Rev. Biochem*., 72, **2003**, 137
[2] G. Paumgartner, *J. Dig. Dis*, 11, **2010**, 119

ANA-PO-20 GLUCOSE OXIDASE IMMOBILIZED IN POLYVINYL ALCOHOL FILM FOR ANTIBACTERIAL SYSTEMS

D. Chirizzi[1], M.R. Guascito[1], C. Malitesta[1], L. Stabili[2]
[1] Laboratorio di Chimica Analitica, Dipartimento di Scienza dei Materiali, Università del Salento, Via Monteroni, 73100 Lecce, Italy.
[2] Istituto per l'Ambiente Marino Costiero CNR U.O.S. di Taranto, Via Roma 3, 74100 Taranto, Italy
maria.rachele.guascito@unisalento.it

The development of new devices based on biocide surfaces has been an intangible objective for many decades. Nowadays scientists need to carry out materials with a surface that has a very broad spectrum of biocidal activity, that can be used repeatedly and that kills via a mechanism which will not result in the emergence of resistant strains [1].
In this work a novel antibacterial system based on immobilization of Glucose oxidase enzyme (GOx) in a poly(vinyl alcol) (PVA) film is presented. PVA represents an ideal enzyme immobilization material because the abundance of hydroxyl groups provides a microenvironment similar to the enzyme's natural environment [2]. It has been in fact widely used because of its inherent good biocompatibility and desirable physical properties, such as elastic nature and good film-forming property [3]. The GOx-PVA composite material has been extensively characterized by UV-vis and X-ray photoelectron (XPS) spectroscopy, to verify the preservation of the enzyme structural integrity and of the enzymatic activity in PVA membrane. Moreover, XPS characterization, revealed a homogeneous film whose structure is not altered under operative conditions.
The antibacterial lysozyme-like activity of GOx-PVA was evaluated by a standard assay on Petri dishes employing *Micrococcus luteus* cell walls. GOx-PVA showed a lysozyme-like activity with a maximum at pH 6.0 and I=0.175. Lysozyme represents the best characterized enzyme involved in the defence against bacteria. This enzyme dissolves certain bacteria by cleaving the B1-4 bonds between N-acetylglucosamine and N- acetylmuramic acid of bacterial cell walls. Thus the findings from this study have implications for future investigations related to employment of GOx-PVA as a compound of pharmaceutical and technological interest.

[1] G. Amitai, J. Andersen, S. Wargo, G. Asche, J. Chir, R. Koepsel, A. J. Russell, Biomaterials 30 (2009) 6522.
[2] Xu Z.A., Chen X., Dong S.J., Trends Analytical Chemistry 25 (2006) 899.
[3] M. Masuda, in: Polyvinyl Alcohol: Developments, ed. by Finch CA. Wiley, New York, 1992.

ANA-PO-21 Skin penetration of gold nanoparticles: a new analytical approach using the synchrotron radiation computed microtomography

G. Adami[a], M. Crosera[a], G. Ceccone[b], E. Larsson[c], G. Tromba[c], T. Brochard[d], A. Bravin[d], M. Bovenzi[e], F. Filon Larese[e]

[a]Dipartimento di Scienze Chimiche e Farmaceutiche, University of Trieste, Via L. Giorgieri 1, 34127, Trieste, Italy
[b]European Commission JRC, Institute for Health and Consumer Protection, Ispra
[c]Sincrotrone Trieste SCpA, Basovizza, Trieste, Italy
[d]European Synchrotron Radiation Facility (ESRF), Grenoble, France
[e]Dip. Clinico di Scienze Mediche, Chirurgiche e della Salute, Univ. of Trieste
Corresponding author: gadami@units.it

The potential for AuNPs to penetrate the skin lies at the centre of the debate concerning the safety for their use and, on the other hand, the possibility of biomedical applications [1]. In a recent study a high concentration of Au in the skin was found after a 24 hour exposure to 12 nm AuNPs suspension in an in vitro test with the Franz diffusion cell method [2].

The present work aims to identify the distribution of gold into the skin using synchrotron radiation computed micro-tomography (μ-CT). Human skin samples were exposed to a 5 nm-AuNP suspension for 24h, fixed in formaldehyde and analysed at the SYRMEP beamline, Sincrotrone Elettra (Trieste, Italy) at an energy of 14.8 keV (proposal n° 20095290) and at the ID17 beamline of ESRF (Grenoble, France) at an energy of 80.7 keV (proposal n° MD-529).

After the tomographic reconstruction, areas of high absorption were identified in the skin samples exposed to AuNPs. These areas have the size and shape of the hair follicles (Fig. 1) and the result seems to confirm the hypothesis that the hair follicles should be the main route of the nanoparticle skin penetration process.

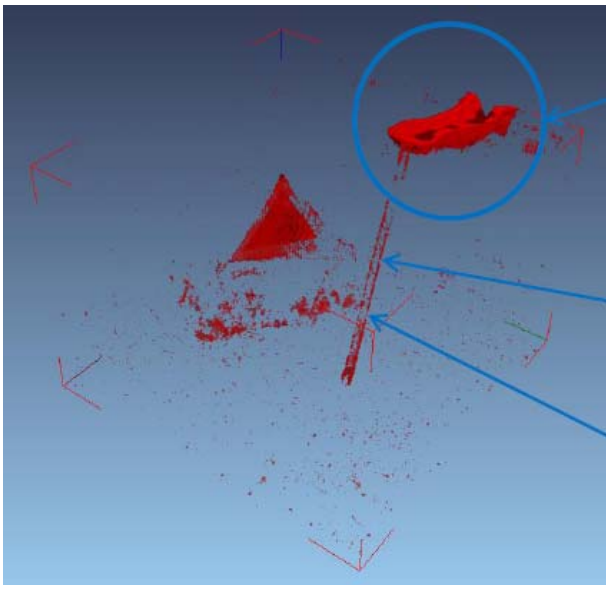


Fig. 1: A rendered 3D volume of the acquired μ-CT slices: a threshold was applied in order to distinguish between skin tissue and high absorbing AuNPs. The arrows and the circle indicate the hair follicles.

[1] M.Crosera, M.Bovenzi, G.Maina, G.Adami, C.Zanette, C.Florio and F.Larese Filon *Int Arc Occ Env Hea* **2009** 82:1043–1055.
[2] F.Larese Filon, M.Crosera, G.Adami, M.Bovenzi, F.Rossi, and G.Maina *Nanotoxicology* **2011** Posted on line (doi:10.3109/17435390.2010.551428).

ANA-PO-22 Simultaneous determination of meropenem, ciprofloxacin, and ofloxacin in human plasma using RP-HPLC-DAD: method development, validation and optimization of various experimental parameters

Carlucci Giuseppe, Settimio Margherita, Locatelli Marcello

Dipartimento di Scienze del Farmaco,Facoltà di Farmacia - Università degli Studi "*G. D'Annunzio*" Chieti-Pescara, Via dei Vestini 31, 66100 Chieti - Italy.

Infections by multidrug-resistant (MDR) *Pseudomonas aeruginosa* are a serious threat in the nosocomial environment often associated with considerable mortality. The type of management for such infections is a matter of debate. Often a combination of one β-lactam agent with an aminoglycoside or fluoroquinolone is suggested as the empirical treatment of choice, but the true effectiveness of that combination is doubtful. Meropenem is a semi-synthetic antibiotic that exhibits significant activity against important aerobic and anaerobic pathogens. Its chemical structure is shown in figure 1A.

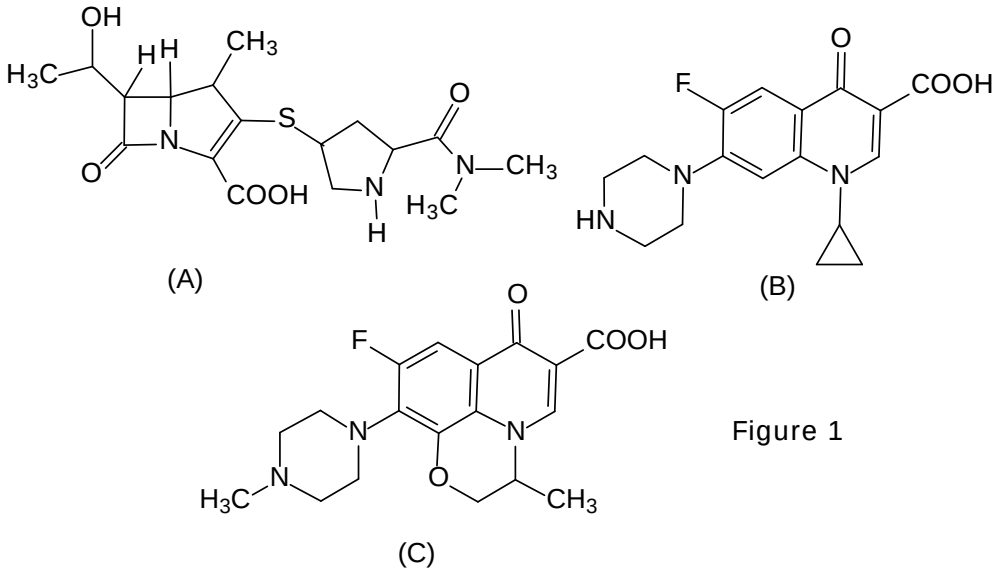


Figure 1

Meropenem is resistant to hydrolysis by bacterial β-lactamases. Ciprofloxacin (figure 1B), is the most potent quinolone against Gram-negative bacteria. Ofloxacin (figure 1C), is effective in treating a variety of acute and chronic infections. The target of the highly selective action of these fluoroquinolones is bacterial DNA-gyrase, a type of topoisomerase II.

A HPLC method with DAD detection was developed optimized and validated for the simultaneous quantification of meropenem, ciprofloxacin and ofloxacin in human plasma. The best resolution was achieved with a

Zorbax Extend (150 x 4.6 mm I.D., 5 μm) column using a gradient mobile phase. Solid-phase extraction were used for sample preparation. Effect of different experimental parameters and various particulate columns on the analysis of these analytes was evaluated.

ANA-PO-23 PAHs determination in rain water: optimization of a HPLC/fluorescence modified method and its application for an environmental monitoring

M. L. Antonelli [a], A. Bacaloni [a], R. Preti [b], G. Vinci [b]

[a] Dipartimento Chimica, Sapienza Università, p.le A. Moro, 5, 00185 Roma-I

[b] Dipartimento Management e Tecnologie, Sapienza Università, via del Castro Laurenziano, 9, 00161 Roma–I

marta.antonelli@uniroma1.it

The Italian regulations, actually in use, provides for the *benzo(a)pirene* determination in atmosphere as unic indicator of the PAHs quantities in air. In fact the actual reference method, indicated by the D. Lgs. Agoust 13th 2010, n. 155 (actuation of the European Directive 2008/50/CE) refers to the law UNI EN 15549/2008, which indicates only the sampling and measurement of the PAHs in air. This methodolgy seems very poor, because of the variable ratios values among the different PAHs present in the urban atmosphere particulate.

The HPLC/fluorescence method here outlined could become a useful and reliable alternative to be proposed for a new and better regulation to the European Community. The method makes use of an HPLC instrumentation (Shimadzu LC-10 ATVP) equipped with a fluorimeter (Shimadzu RF-10AXL), and different chromatographic columns and pre-columns have been tested to optimize the results to obtain the best separation and the minimum time of analysis. To be noticed the particular clean-up method [1,2] used, which allows very concentrated samples to be injected into the HPLC system. By means of the proposed method the determination of 16 PAHs in rainwater samples [3], collected in different sampling stations of the Rome district, are in progress and they will performed over at least 1 year period of time. The PAHs content in raiwater seems a good information regarding the PAHs pollution in the urban atmosphere, even if the season time, the weather and also temperatures must be taken into account for each sample. The proposed method could be a useful method not only with respect to the humid depositions, but also to resolve the poorness of the *benzo(a)pirene* single determination as it is recommended by the European Directives.

[1] L. Zoccolillo, L. Amendola, G.A. Tarallo, *Int. J. Anal. Chem., 63*, **1996**, 91

[2] A. Bacaloni, L. Zoccolillo et Al., *Ann. Chim., 94*, **2004**, 751

[3] Jun He, Rajasekhar Balkasubramanian, *Chemosphere, 78*, **2010**, 742.

ANA-PO-24 Simultaneous determination of salicylates and benzoic acid in food by SPME-LC-UV-DAD

A. Aresta, G. Lioce, C.G. Zambonin

Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, via Orabona 4, 70126 Bari
a.aresta@chimica.uniba.it

Salicylate, acetylsalicylic acid (aspirin) and their derivatives (salicylates) are used as fungicidal and antimicrobial agents in pharmaceuticals preparations (external use) as well as in the treatment of inflammatory processes as antipyretic and analgesic drugs (internal use).
Salicylates have been also used in beverages and foods for preservation, but it has been forbidden since the sixties in several countries due to its toxicity [1].
These chemicals occur also naturally in many plants, including many fruits vegetables, and herbs. Salicylates in plants act as a natural immune hormone and preservative, protecting the plants against diseases, insects, fungi, and harmful bacteria.
Salicylates are generally regarded as safe for adults, even if high enough doses are harmful to everyone. However, most people can handle average amounts of salicylate in food, products and medications without any adverse affects on their health. Unfortunately, there is a small percent of the population for which even a small dose of salicylates can be a problem. Some adults and children may develop symptoms and health problems from salicylates which are dose-related. This is called 'Salicylate Sensitivity' or 'Salicylate Intolerance'.
Individuals that are sensitive to salicylates may suffer with urticaria, angiooedema, rhinitis, bronchial asthma and recurrent nasal polyps [2]. The chronic nature of some of these clinical presentations, without other obvious cause, may suggest an underlying etiology related to dietary salicylates. A low salicylate diet may be of clinical benefit to such affected individuals. This cannot be established however, until the salicylate content of different food and drinks is known. Data on the salicylate content of foods are scarce and contradictory. Our aim was to develop an accurate analytical method to measure the salicylates content of selected food commodities.
Existing papers on this topic have been essentially based on chromatographic techniques, after purification of the analytes by means of complicate isolation procedures. A good alternative could be represented by the use of solid-phase microextraction (SPME), a solventless technique initially coupled to GC and later interfaced also to LC [3].
Thus, in the present work, a solid-phase microextraction (SPME)–LC–UV-DAD method for the simultaneous determination of salicylates and benzoic acid in food samples was developed for the first time using a

polydimethylsiloxane/divinylbenzene (PDMS/DVB) coated fiber. The procedure required very simple sample pretreatments, isocratic elution, and provided highly selective extractions. The applicability of the method was demonstrated on different food products, i.e. kiwis, blueberries, lemons, mandarins, oranges, broad beans, and commercial fruit juices.

[1] T.M. Brown, A.T. Dronsfield, P.M. Ellis and J.S. Parker, *Educ. Chem.*, 35, **1998**, 47-51.
[2] S. Jantti-Alanko, E. Holopainen, and H. Malmberg, *Rhinology*, 8, **1989**, 59–64.
[3] C.G. Zambonin, *Anal Bioanal. Chem*., 375, **2003**, 73–80.

ANA-PO-25 Characterizing parameters of Chocolate.

Emilio Bottari, Francesco Di Pasqua, Maria Rosa Festa, Simone Mazzone
Dipartimento di Chimica, ed. Cannizzaro, Università "La Sapienza", Piazzale A. Moro 5, 00185 Roma
e-mail: emilio.bottari@uniroma1.it

The protein nitrogen and the metallic components were evaluated to characterize chocolate. Ten marks with a percentage of cocoa generally in the range 70 – 100% were analyzed. Only one was 50%. In one case the declared origin of cocoa was Venezuela. The protein nitrogen was determined by applying the Kjeldhal procedure. The percentage of the product (i.e. % of cocoa) per protein nitrogen was in the range 0.7 – 1.2, except, as expected, for the sample with 50%. In this case the percentage was 0.33.

As some cations are toxic and others can have nutritional or toxic effect depending on their concentration, total arsenic, cadmium(II), lead(II), copper(II), zinc(II) and total selenium were determined. To eliminate the organic components, weighed samples of chocolate of different firms were ignited at 900°C to obtain ashes at constant weight. The range of the percentages of ashes was within 1.4 and 3.7%. Ashes were dissolved in HCl and the content of the above cations was determined by means of Atomic Adsorption (AA) spectrometry. Cadmium(II), copper(II) and lead(II) were determined in flame acetylene – air (or in furnace on the basis of the concentration), whereas total arsenic, total selenium and mercury(II) were determined by using the hydride kit and treating the samples with sodium bore hydride and 10% HCl.

Concerning the cation concentration, it was observed that cadmium(II) and lead(II) are present at level less than 1 ppm (mg/kg) and then their analysis had to be carried out by using the graphite furnace. The concentration of zinc(II) and copper(II) was more than 5 ppm, often it reached 30 ppm. Zinc(II) was often double than copper(II). Copper was in the range 6 – 18 ppm, whereas zinc (II) was in the range 15 – 30 ppm.

Cations analyzed by the hydride kit were present in very different amounts, but in the range of ppb (μg/kg) quantities. Mercury is present generally at about 1 ppb, except for one sample where was about 4 ppb. Higher values can be observed for arsenic present in the range within 7 – 31 ppb. The selenium concentration showed a large variability, because it was present within 3 and 50 ppb. Only one sample contains 100 ppb of selenium. It means that a range of about 100 ppb is covered.

By taking in account that the cocoa percentage are different for the analyzed samples, the found quantities of arsenic can be considered near. However, such quantities are higher than the generally accepted value, about 0,2 ppb.

The values relative to selenium are acceptable, even advantageous for the alimentary diet. Only one sample containing 100 ppb of selenium can be dangerous. It seems interesting to observe that the same chocolate sample with 100 ppb of selenium, contains high quantities also of the other cations, so that it can be considered the worst sample.

ANA-PO-26 Formulation and characterization of new packaging material incorporating chitosan nanoparticles-vitamin E and C for food shelf life improvement

Calvano C.D.,[a] Trapani, G.,[b] Barnaba M.,[a] De Giglio E.,[a] Cafagna D.,[a] Aresta A.,[a] Zambonin C.G.[a, c]

[a] Dipartimento di Chimica [b]Dipartimento Farmaco Chimico and [c]Centro Interdipartimentale SMART, Università degli Studi di Bari Aldo Moro, via Orabona 4, 70126 Bari
calvano@chimica.uniba.it

One of the principal causes of food quality deterioration is the oxidation of unsaturated lipids initiated by free radicals. When lipids are exposed to environmental factors such as air, light and temperature, oxidation reactions start to produce undesirable flavours, rancid odours, discoloration and other forms of spoilage. During oxidation, a series of volatile and non-volatile compounds are originated, altering sensory proprieties, causing rancid flavors and decreasing the nutritional quality and safety, which may play a role in the development of some diseases [1]; in addition lipid oxidation could cause a consumer rejection of the food product.
The rate of autoxidation depends on temperature, pH, the degree of unsaturation of the fatty acids and the number of unsaturated fatty acids in the triacylglycerol or phospholipid molecule, as well as on the availability of oxygen and transition metal ions. The other major cause of food spoilage during storage is the bacterial contamination arising from several sources, such as the animal itself, the external environment and its handling.
Packaging is important to preserve the quality and safety of fruits, vegetables and processed foods for assured shelf life extension. The new generation of edible coatings is especially designed to allow the incorporation and/or controlled release of antioxidants, vitamins, nutraceuticals, and natural antimicrobials [2-4].
Among packaging material, chitosan has been well-known for its excellent film-forming property, antimicrobial activity, and unique coagulating ability with metal and other lipid and protein complexes. It seems to be due to the presence of high density of amino groups and hydroxyl groups in the chitosan polymer structure. Its high binding ability and antimicrobial properties are both beneficial in developing new applications of this natural polymer in food preservation. Herein, we aimed at the formulation of chitosan-cyclodextrin nanoparticles (NPs) obtained by the ionic gelation process in order to load vitamin E and C selected as powerful antioxidant agents. In fact, vitamin E is important in the prevention of lipid peroxidation, whereas vitamin C reacts effectively with superoxide and hydroxyl radicals. Vitamins C and E and vitamin precursors (e.g. carotenoids) should reduce the rate

of initiation or prevent the propagation of free radicals as notable non-enzymatic antioxidants.
The NPs will be characterized in terms of light scattering, zeta-potential, vitamins content and by X-Ray Photoelectron Spectroscopy (XPS) measurements. Afterwards, the NPs loading vitamin C and E will be dispersed on polymeric films that are usually found as food packaging materials.

[1] R. M. Adibhatla, J.F. Hatcher *Antioxidants & redox signaling,* 12, **2010**, 125-169
[2] M. Vargas, C.Pastor, A.Chiralt, D. J. McClements, C. González-Martínez *Crit Rev Food Sci Nut*, 48, **2008**, 496–511.
[3] P. K Dutta, S. Tripathi, G. K. Mehrotra, J. Dutta, *Food Chem* 114, **2009**,1173-1182.
[4] P.E. Chiu, L.S. Lai, *Int J Food Microbiol* 139**, 2010,** 23-30.

ANA-PO-27 Phenolic content and radical scavenging ability of wild fruits of *Rubus* species and related jam and seeds from Calabria

Alessia Fazio, Bartolo Gabriele, Pierluigi Plastina

Department of Pharmaceutical Sciences, University of Calabria, 87036 Arcavacata di Rende (CS), Italy
a.fazio@unical.it

Small berries are rich sources of bioactive compounds such as flavonoids, phenolic acids and vitamin C, which are known to display potential health-promoting effects [1]. Blackberry is an edible fruit produced by several species of the *Rubus* genus of the *Rosaceae* family. In this study, we have determined the chemical composition, the phenolic content, and the antioxidant activity of Southern Italy blackberries (*Rubus ulmifolius* Schott) growing wild in Calabria. In particular, the studies were extended to two anatomically distinct parts of fruit, the pulp and the seeds and a derived product such as jam. The fruits were picked randomly from different parts of wild bushes on mountain slopes at an altitude of 1000 m above sea level (C.da Pallone, Cosenza). The freeze-dried fruits were crushed in a mortar and were sieved using a 60 mesh screen to achieve the separation of seeds from the pulp. One part of the pulp was directly analyzed, while another part was cooked to make jam. Total lipids were extracted from ground seeds (5 g) with hexane at 90°C for 2 h (22% yield w/w). The fatty acid composition was then determined by GLC after a direct transesterification procedure [2] carried out in methanol-benzene with acetyl chloride. The most represented fatty acids were linoleic and linolenic acids (89,6%). The methanolic extract of defatted seed flour showed a strong radical scavenging activity determined using DPPH test (97%). On the other hand, the antioxidant activity of two phenolic fractions extracted from the pulp (ethyl acetate extract containing phenolic acids and flavonol glicosides, and acidic methanol extract containing anthocyanins) was lower than that of seeds (70% and 69% respectively). The processing of the berries into jam, prepared by cooking 50 g of pulp with 25 g of sugar and 1,25 g of pectins for 3 min, led to a significant loss of radical scavenging activity (50 %). HPLC-UV/vis and HPLC-ESI analyses were used to determine anthocyanin and phenolic composition. The results indicated that cyanidin-3-glucoside was the major anthocyanin in the pulp while the most abundant non-anthocyanin phenolic was epicatechin. The main phenolic compound detected in the metanolic extract of the seeds was free ellagic acid.

[1] J. Tabart, C. Kevers, J. Pincemail, J. O. Defraigne and J. Dommes, *J. Agric. Food. Chem., 54,* **2006**, 6271.
[2] G. Lepage and C. C. Roy, *J.Lipid Res.*, *27*, **1986**, 114.

ANA-PO-28 Determination of very low levels of 5-(hydroxymethyl)-2-furaldehyde (HMF) in natural honey: comparison between the HPLC technique and the spectrophotometric White method.

Truzzi Cristina[a], Illuminati Silvia[a], Annibaldi Anna[a], Finale Carolina[a], Rossetti Monica[b], Giuseppe Scarponi[a]

[a]Department of Marine Science, Università Politecnica delle Marche, Italy.

[b]ASSAM (Agenzia Servizi Settore Agroalimentare delle Marche), Centro Agrochimico Regionale, Jesi (An), Italy.

c.finale@univpm.it

HMF (5-hydroxymethyl)-2-furaldehyde is a molecule of interest both as a marker of quality deterioration, caused by excessive heating or inadequate storage conditions, and for its cancerogenic effects. The European Union [1] established that its concentration in honey (except baker's honey and in products from regions with tropical climate) should not exceed 40 mg kg^{-1}. The International Honey Commission [2] reports three official methods for the analysis of HMF in honey, two spectrophotometric methods, determinations after White [3] and after Winkler [4], and an HPLC method [5], however the Winkler method has been abandoned because of the toxicity of the reagents used.

In this work we have compared the official methods still in use (the White method and the HPLC method) for the determination of HMF in unifloral honeys (acacia, chestnut, coriander, linder, sunflower) and honeydews with very low HMF contents (< 4 mg kg^{-1}), i.e. the most critical determinations, in terms of concentration results of samples and precision of the method.

For concentrations in the range 1-4 mg kg^{-1} the HMF values obtained with the two methods are comparable both for unifloral and honeydew samples. For samples with HMF content < 1 mg kg^{-1}, generally results significantly in excess were obtained with the spectrophotometric method in comparison to the HPLC method. In particular, 20-30% higher for samples with HMF content in the range 0.5-1 mg kg^{-1}, ~70% higher for samples with HMF content < 0.5 mg kg^{-1}. As regards precision, the HPLC method (±3.3% as RSD) is better than the White method (±6.4%) for unifloral honey samples, except for the chestnut honey and for honeydew samples, where precision is comparable for the two methods, about ±10% and ±7-9%, respectively.

[1] European Council Directive 2001/110/EC of 20 December 2001, Off J Eur ***Communities***, 12.1.2002.

[2] IHC, Bogdanov, S., (1999) Harmonized methods of the International Honey Commission. Swiss Bee Research Centre, Bern

[3] White, J., 1979. Journal AOAC 62, 509-514.

[4] Winkler, O., 1955. Z Lebensm Unters F, 102(3), 161-167.

[5] Jeuring, J., Kuppers, F., 1980. Journal AOAC 63, 1215-1218.

ANA-PO-29 Sorption and Photodegradation Studies of Marbofloxacin and Enrofloxacin on Clays

Federica Maraschi[a], Michela Sturini[a], Andrea Speltini[a], Elisa Rivagli[a], Antonella Profumo[a], Massimo Setti[b] and Angelo Albini[a]

[a]Department of Chemistry, University of Pavia, via Taramelli 12, 27100 Pavia (Italy)
[b]Department of Earth Sciences and Environmental, University of Pavia, via Ferrata 1, 27100 Pavia (Italy)
federica.maraschi@unipv.it

Marbofloxacin (MAR) and Enrofloxacin (ENR) are two largely employed veterinary Fluoroquinolones (FQs) in the South Lombardy plain. Their occurrence and fate have been already investigated in natural waters [1] and agricultural soils [2,3], where they can be accumulated up to mg kg^{-1} levels as a result of manuring [4]. Their strong adsorption makes them persistent pollutants. This study focused on adsorption and interaction of FQs on three different clay minerals, montmorillonite, kaolinite and sepiolite. Adsorption/desorption behavior depends on pH of the medium, and only in the case of montmorillonite, both MAR and ENR cause an expansion of the spacing between layers (from XRD analysis). As clay percentage plays a significant role in the FQs soil adsorption, an influence on their photodegradation rate is expected too. Photodegradation experiments are ongoing on clays spiked with each antibiotic (mg kg^{-1}). Results will be compared to those obtained from typical agricultural soil collected near Pavia [2].

[1] M. Sturini, A. Speltini, F. Maraschi, A. Profumo, L. Pretali, E. Fasani and A. Albini, *Environ. Sci. Technol.*, *44*, **2010**, 4564.
[2] A. Profumo, M. Sturini, A. Speltini, F. Maraschi, L. Pretali and E. Fasani, Determination of trace veterinary fluoroquinolones in soils and waters and role of photochemistry in their degradation. Presented at Euroanalysis XV, Innsbruck, September 6-10, **2009**.
[3] M. Sturini, A. Speltini, F. Maraschi, E. Rivagli and A. Profumo, *J. Chromatogr. A*, *1217*, **2010**, 7316.
[4] A. Speltini, M. Sturini, F. Maraschi, A. Profumo and A. Albini, *Trends Anal. Chem.* **2011** in press.

ANA-PO-30 Development and validation of a GC/ECD method for analysis of PCBs in milk

V.Nardelli,[a] F.Casamassima,[a] D.Centonze,[b] M.Cristino,[a] D. dell'Oro,[a] G.Gesualdo,[a] C.Palermo.[b]

[a] Istituto Zooprofilattico Sperimentale Puglia e Basilicata Via Manfredonia n° 20 - 71121, Foggia, Italy

[b] Dipartimento di Scienze Agro-Ambientali Chimica e Difesa Vegetale e Bioagromed-Università degli Studi di Foggia, Via Napoli, 25 -71122, Foggia, Italy

v.nardelli@izsfg.it

PCBs are synthetic organic compounds characterized by their toxicity and persistence in the environment, and for this reason in 2001 PCBs were banned by the Stockholm Convention on Persistent Organic Pollutants. PCBs can be transported long distances, and have been found in places which are far from where they were manufactured or used. They have been detected in all indoor and outdoor environmental media (surface and ground water, soil and food).Since these compounds exhibit a high affinity for lipophilic matrices, they can also accumulate in milk of animals, which eat PCB-contaminated feed.The aim of the present work was the development and validation of a method based on a simple cleanup procedure and a GC/ECD determination of 6 PCB-NDL (28,52,101,153,138,180) in milk samples. Several purification procedures involving sulphuric acid treatment and different solid phases extraction, [1] were tested in order to minimize interferences and to achieve high recovery values. Performance parameters, such as precision, specificity, ruggedness, linear range, LOD, LOQ, for the method validation were determined by the GC/ECD analysis of standard solutions and spiked samples in the range 0,5-4 ng g^{-1} containing 10 μg□L^{-1} PCB 209 as the internal standard. The GC experimental conditions were optimized to allow an improvement of the time and the chromatographic resolution. Calibration curves of the investigated PCBs were obtained by using three replicate injections of the standard solution at five calibration levels, from 1 to 40 μg L^{-1} Calibration curves were linear over the whole range of concentrations tested for all congeners, as indicated by the very good values of the determination coefficients ($r^2 \geq 0.99$). LODs and LOQs were in the range 0.03-0.09 and 0.10-0.27 μg L^{-1}, respectively. The only use of Bond Elut PCB [2] gave short cleanup times, less viscous solutions, with few interferent compounds and clean extracts with good recoveries (71-111%) and CV% (3,3-18,0%), lower than reference value (23%) derived from Thompson's equation. [3]

[1] V. Nardelli, C.Palermo, D.Centonze *J. Chromatogr., A 1034* ,**2004**, ,33-40.

[2] V. Nardelli, D.dell'Oro, C.Palermo, D.Centonze *J. Chromatogr., A 1217* ,**2010**, 4996-5003.

[3] M.Thompson. *Analyst 125*, **2000**, 385

ANA-PO-31 Optimization and validation of a multi-matrix confirmatory method for the determination of Nicarbazin by HPLC-Diode Array Detection

Marilena Muscarella[a], Antonio Armentano[a], Marco Iammarino[a], Donatella Nardiello[b], Carmen Palermo[b], Diego Centonze[b]

[a] Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, via Manfredonia 20, 71121 Foggia, Italy

[b] Dipartimento di Scienze Agroambientali, Chimica e Difesa Vegetale, Università degli Studi di Foggia, via Napoli 25, 71122 Foggia, Italy

m.muscarella@izsfg.it

Nicarbazin is an authorised feed coccidiostat that is used for chickens for fattening. The actual feed production technologies cause unavoidable cross-contamination in feed for non-target animal species with possible repercussions on public health (1). Recently two European Union rules (Directive 2009/8/EC L 40/19 of 11.02.2009 and Regulation 2009/124/EC L 40/7 of 11.02.2009) have been issued, in which the nicarbazin maximum levels in non-target feed and foodstuffs of animal origin were reported. The development of a suitable method for official controls is necessary to reach these Maximum Residue Limits (MRL) for nicarbazin in feed and animal derived food.

In this work a sensitive, accurate and fast multi-matrix confirmatory method was optimized and validated for quantitative determination of nicarbazin in egg, liver, muscle and feed matrices. The method is based on high performance liquid chromatography and diode-array detection. The sample preparation requires a rapid extraction with acetonitrile and a purification step by *n*-hexane. A sensitive detection was obtained by using an absorbance wavelength of 350 nm and an exploration wavelength range 200-420 nm. Under optimized experimental conditions, nicarbazin separation was obtained in less than 20 minutes, using a C18 column eluted with a mixture of acetonitrile/water 50/50 (v/v) at a flow rate of 1.0 mL min^{-1}. The excellent analytical parameters (see table) of accuracy, detection and quantification limits and measurement uncertainty (%) were evaluated by following a validation procedure indicated in the European guidelines of Regulation 882/2004/EC (2) and Decision 657/2002/EC (3).

Sample	Repeatability RSD (n=6)	Reproducibility RSD (n=18)	Recovery % (n=18)	LOD (µg kg^{-1})	LOQ (µg kg^{-1})	Measurement uncertainty (%)
Eggs	3.4	4.0	75.2	8.6	26.2	11.1
Liver	1.2	4.0	80.0	8.6	26.2	11.1
Muscle	1.3	2.4	87.0	4.1	12.3	6.9
Feeds	3.3	3.7	74.4	3.7	11.2	6.6

[1] Panel on contaminants in food chain, *The EFSA Journal, 690*, **2008**, 1-34.

[2] European Commission. *Official Journal of the European Union, L165*. **2004**. 1–141.

[3] European Commission. *Official Journal of the European Union, L221*. **2002**. 8–36.

ANA-PO-32 The potentialities of capillary electrophoresis for the characterisation of wheat germplasm. A case study.

Piergiovanni A.R.,[a] Porfiri O.,[b] Fiorani M.[c], Pignone D.[a]

[a] Istituto di Genetica Vegetale-CNR, via Amendola 165/a, Bari, Italy
[b] Agronomo, Dottore di Ricerca, Urbisaglia (MC)
[c] Prometeo s.r.l., Urbino
e-mail angelarosa.piergiovanni@igv.cnr.it

In response to market demand, the interest toward old landraces of wheat, legume etc. surviving *on-farm* in marginal areas of Italy is constantly increasing. The survival of this germplasm is related to its characterisation and discrimination from modern varieties. Methods based on morphological plant traits or on DNA-based analyses are expensive and time demanding. Conversely, biochemical analyses are cheap and require short time. Gliadins, one of the most abundant storage protein of the caryopsis, is a very useful tool in the discrimination and characterisation of cereal accessions. Capillary electrophoresis (CE) allows automated and fast electrophoretic analysis of gliadins [1]. Presently, the literature on Italian landraces belonging to Oriental wheat (*T. turanicum* Jakubz.) is scarce. This study was undertaken to investigate by CE, the genetic similarity between some Oriental wheat samples gathered from Italian farmers and the accession QK-77, registered as Kamut®.

Nine samples acquired from farmers, seed trade companies and USDA gene-bank (PI278350, collected in Italy) were analysed. They were designated with different names such as 'Saragolla', 'Kamut', 'Farro lungo', 'Grano del faraone' and durum wheat. Kamut® was included as a reference. Gliadins were extracted from single caryopsis, 10 seeds per sample were analysed. CE analyses were carried out as previously described [2]. The samples were cultivated in an experimental field located at Urbisaglia (MC) to record the main morphological plant traits.

A very low intra-population variation of gliadin profiles was observed. The comparison of gliadin profiles among samples evidenced a very high similarity of profiles with the exception of 'Saragolla di Abbateggio'. Plant morphological traits agreed with electrophoretic analyses. These findings suggest that in Italy there are wheat landraces genetically very similar to Kamut®, though they are indicated with different vernacular names. It can be inferred that these landraces were originated by the same pool of seeds. The efficacy of CE as a fast and cheap method for the study of genetic relationships among wheat germplasm has been evidenced by the agreement of morphological and electrophoretic data.

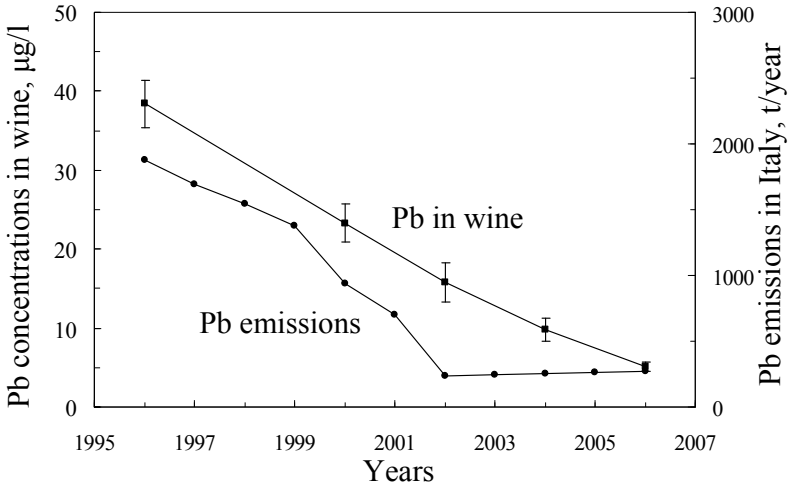
[1] S.R.Bean and G.L.Lookhart, *J.Agric.Food Chem.*, 48, **2000**, 344.
[2] A.R.Piergiovanni and N.Volpe, *Cereal Chem.*, 80, **2003**, 269.

ANA-PO-33 Recent temporal variations of trace metal contents in wine.

S. Illuminati,[a] A. Annibaldi,[a] C. Finale,[a] C. Truzzi,[a] G. Scarponi[a]

[a]Dipartimento di Scienze del Mare, Università Politecnica delle Marche, Via Brecce Bianche, 60131, Ancona
s.illuminati@univpm.it

In a previous work, voltammetric stripping determination of Cd, Pb and Cu in wine (after UV digestion) was set up by us using the square-wave technique (SWASV) for the first time [1,2]. A thin mercury film electrode was used, applying the following parameters: E_{dep} = –950 mV (Cd), –750 mV (Pb, Cu) *vs*. Ag/AgCl, 3 M KCl; SW scan from –950 mV (Cd), –750 mV (Pb, Cu) to + 30 mV; E_{SW} = 20 mV; f = 100 Hz; ΔE_{step} = 8 mV, t_{step} = 100 ms, t_{wait} = 60 ms, t_{meas} = 3 ms.



In this work, a study of the recent temporal trends of the three metal concentrations was carried out, also to evaluate the potential relationship between the Pb content in wine and the removal of the metal from gasoline (which has been operative in Italy since 2002). The white wine *Podium* (a 100% *Verdicchio* wine) was used. This wine keeps well and samples of vintages between 1996 and 2006 were available. The results show that the Pb content in wine decreased from 38.4 µg/L in 1996 to 5.1 µg/L in 2006, a reduction of ~80%. This can be related to the recent phasing out of Pb from gasoline in Italy, which led to a similar decreasing trend of Pb emissions in the atmosphere (see Figure). Note that the current legal limit for Pb is 200 µg/L [3] (OIV limit 150 µg/L [4]), a considerably higher value than those measured here. By contrast, Cd and Cu do not show significant variations in the same period, with average measured values of: Cd ~0.3 µg/L (OIV limit 10 µg/L [4]) and Cu 50 µg/L (legal and OIV limits 1000 µg/L [4,5]).

[1] S. Illuminati, A. Annibaldi, C. Truzzi, G. Scarponi. Proceed. XXII National Meeting on Analytical Chemistry, Como (CO), 12-16/09/2010.
[2] S. Illuminati, A. Annibaldi, C. Truzzi, G. Scarponi. Determination of Cd, Pb and Cu in wine: optimization of the sample pre-treatment procedure and the voltammetric (SWASV) analysis. *In preparation*.
[3] European Commission Regulation (EC), no. 1881/2006 of 19 December 2006.
[4] OIV, *Compendium of International Methods of Wine and Must Analysis*, Vol. 2, 2011, Annex C, p. OIV-MA-C1-01.
[5] Ministry Decree 29/12/86, published in Italian OJ no. 13, 17 January 1987.

ANA-PO-34 UV-A and Visible Photolytic and Photocatalytic Degradation of Aqueous Fluoroquinolones: Reaction Kinetics and Byproducts

Michela Sturini, Andrea Speltini, Federica Maraschi, Luca Pretali, Antonella Profumo, Elisa Fasani and Angelo Albini

Department of Chemistry, University of Pavia, via Taramelli 12, 27100 Pavia (Italy)
michela.sturini@unipv.it

Fluoroquinolones (FQs) are antibacterial agents employed in human and veterinary medicines, especially in animal breeding, and they have been widely detected in environmental waters [1], indicating their ineffective removal by conventional wastewater treatment plants [2].
Photodegradation is actually a significant removal pathway in water systems [3,4]. In this study we investigated the photolytic and titanium dioxide (TiO_2) photocatalytic degradation using UV-A, visible and natural solar light, in ultrapure and river water. Different FQs have been examined, i.e. Ciprofloxacin, Danofloxacin, Levofloxacin, Enrofloxacin, Marbofloxacin and Moxifloxacin, the last ones belonging to the most recent generation. Experiments were carried out at environment-significant concentration (20-50 $\mu g\ L^{-1}$) and at mg L^{-1} levels, and samples have been analyzed by HPLC-UV and FD. First order kinetics were observed both under direct photolysis and with TiO_2. Degradation rates were faster by heterogeneous photocatalysis for all of the drugs, except for Ciprofloxacin. Byproducts from both photocatalysis and photolysis were identified by HPLC-ESI-MS and the chemical paths compared.

[1] A. Speltini, M. Sturini, F. Maraschi and A. Profumo, *J. Sep. Sci.*, *33*, **2010**, 1115.
[2] E.M. Golet, A.C. Alder and W. Giger, *Environ. Sci. Technol.*, *36*, **2002**, 3645.
[3] L. Pretali, E. Fasani, D. Dondi, M. Mella and A. Albini, *Tetrahedron Lett.*, *51*, **2010**, 4696.
[4] M. Sturini, A. Speltini, F. Maraschi, A. Profumo, L. Pretali, E. Fasani and A. Albini, *Environ. Sci. Technol.*, *44*, **2010**, 4564.

ANA-PO-35 Determination of YLOID in soil and grapevine systems (Vitis vinifera L.) by ICP-MS technique: a hopeful proxy for the geographical characterization of food products?

N. Tuzzolino,[a] A. Pisciotta,[b] P. Censi,[a] F. Saiano[c]
[a]DiSTeM, Università di Palermo, Via Archirafi 22, 90123, Palermo, Italy.
[b]Dip.DEMETRA, Università di Palermo, Viale delle Scienze ed 4, 90128, Palermo, Italy.
[c]Dip.S.Ag.A., Università di Palermo, Viale delle Scienze ed 4, 90128, Palermo, Italy.
nicola.tuzzolino@unipa.it

Chemical behavior of YLOID (Y and Lanthanoid) into soil was extensively studied both to evaluate geochemical process. Metal cations can be immobilized onto particle surface of soil due to the formation of stable complexes with organic matter. If environmental conditions change metals can be mobilized and therefore to became bioavailable [1].

In recent years identification of the geographical origin of food has acquired very importance because consumers are more and more interested in knowing the provenance of the food purchased and/or eaten [2]. Then the knowledge of a relationship between the chemistry of the substrates and the food could be an important tool for the quality guarantee of traditional food products.

The uptake of YLOID and their distribution in grapevine system were studied under controlled conditions following the plants growth.

The experimental system consisted of a set of 30 plants, divided into two groups: blank and YLOID. The first group (blank) was used as control, the other was polluted in an unique step with a YLOID concentration of $2.5 \cdot 10^{-3}$ mmol for Kg of substrate (peat and gravel).

Three replicates for each group and for each phenological stage were sampled. To study the metal distribution, the main part of plants: roots, stem, woody and herbaceous shoot and apex were analysed.

The obtained results were critically discussed on the basis of the different amount presents in all parts of plants.

[1] S.M.Ding, T.Liang, C.S.Zhang, J.C.Yan, and Z.L.Zhang, Accumulation and fractionation of rare earth elements (REEs) in wheat: controlled by phosphate precipitation, cell wall absorption and solution complexation, *Journal of Experimental Botany*, 56, **2005**, 2765-2775, and bibliography cited.

[2] S.Spalla, C.Baffi, C.Barbante, C.Turretta, G.Cozzi, G.M.Beone and M.Bettinelli, Determination of rare earth elements in tomato plants by inductively coupled plasma mass spectrometry techniques, *Rapid Communications in mass spectrometry*, 23, **2009**, 3285-3292.

ANA-PO-36 Detection of fraudulent addition of barley to coffee powder by means of NIR spectroscopy and multivariate regression

Heshmatollah Ebrahimi-Najafabadi[a,b], Riccardo Leardi[a], Paolo Oliveri[a], Chiara Casolino[a], Mehdi Jalali-Heravi[b], Silvia Lanteri[a]

[a]Department of Pharmaceutical and Food Chemistry and Technologies, University of Genoa, Via Brigata Salerno (ponte), I-16147 Genoa, Italy
[b]Department of Chemistry, Sharif University of Technology, Tehran, Iran
Email: oliveri@dictfa.unige.it

Coffee characterisation by means of instrumental fingerprints has gained increasing attention as a tool for controlling and avoiding adulterations [1]. The objective of the present study was to verify the feasibility of detecting fraudulent additions of barley to coffee, by means of NIR spectroscopy and multivariate calibration methods. In order to generalise the results, nine different types of grain coffees, including both *arabica* and *robusta* species as well as mixtures, with different roasting degrees, and four different barleys were collected. Beans were grounded and the powders were mixed at 10 different levels, between 2 and 20% (w/w) of barley. Since all the possible combinations of coffee and barley samples at each level would have required 360 mixtures, D-optimal design was applied to select 100 and 30 representative mixtures as calibration and test sets, respectively. NIR spectra of the powder samples were recorded in the reflection mode, in the range 4,000-10,000 cm^{-1} with a 4 cm^{-1} resolution, by an FT near-infrared spectrophotometer based on a polarization interferometer (Buchi NIRFlex N-500). Spectra of the nine pure coffee samples were also added to the calibration set.

Partial least squares (PLS) regression and kernel orthogonal partial least square (KOPLS) techniques [2] were used to build the predictive models. The number of latent variable, orthogonal components and kernel band width were optimised within cross validation cycles. The optimised models were validated by prediction of barley concentration for the samples of the test set. In addition, eleven different mixtures of two different coffee and barley samples (not included in the training samples) were evaluated as an external test set. The root mean square error (RMSE) of the PLS model for the calibration, test and external test sets were 1.2, 1.4 and 1.04, respectively. The corresponding RMSE values of the KOPLS models were 0.63, 0.83 and 0.97. The method presented can be considered as a rapid and reliable tool for detecting the adulteration of coffee by addiction of barley.

[1] I. Esteban-Diez, J.M. Gonzalez-Saiz, C. Saenz-Gonzalez, C. Pizarro, *Talanta 71,* **2007,** 221–229.
[2] M. Rantalainen, M. Bylesj, O. Cloarec, J.K. Nicholson, E. Holmes, J. Trygg, *J. Chemometrics 21,* **2007**, 376–385.

ANA-PO-37 LA-ICP-MS analysis of otoliths: a new prospecting tool of marine metal pollution.

A. Pennetta[a], G. E. De Benedetto[a], A. Di Franco[b], P. Guidetti[b]

[a]Laboratory of Chemical Analyses for Cultural Heritage, Department of Fine Arts and History, University of Salento, 73100, Lecce, Italy
[b]Department of Biological and Environmental Sciences and Technologies, University of Salento, 73100 Lecce, Italy
antonio.pennetta@unisalento.it

Trace elements in otoliths may provide information on dispersal history of marine fishes. We propose the use of the fish otoliths as biomarkers of marine pollution. The longitudinal section of otoliths of fish shows, indeed, daily growth rings whose elemental composition reflects the composition of the waters where the fish has been.

Interest in analytical mass spectrometry associated with laser ablation for sample introduction has increased markedly during the past few years. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) appears to be the only analytical approach for nearly non-destructive determination of a large number of elements with very low detection limits, permitting the characterization of the distribution of trace elements in geological, archaeological and environmental materials. Moreover, laser ablation can be performed with beam diameters ranging from few microns to hundredths microns, allowing both space resolved and 'bulk analysis'.

Space resolved capabilities of LA-ICP-MS have been employed to study the concentrations of trace elements in otoliths to obtain information about the origin of fish and rebuild their migratory flows [1,2]. However otolith matrix can incorporate trace elements from its environment [3] and the findings of elements having environmental concerns in these structure is the signal that fish has experienced polluted waters. As a result LA-ICP-MS provides precise positional information with the potential for chronological studies of otoliths and the history of the environment that the fish have lived in.

In this work the development and the optimization of LA-ICP-MS method and the relevant application will be shown.

[1] P. Guidetti, M. Petrillo, G. de Benedetto, G. AlBertelli, Natal origin of European anchovy: a case study in the Ligurian Sea (NW Mediterranean), *Biol. Mar. Mediterr.*, 17, **2010**, 149
[2] A. Di Franco, G. De Benedetto, G. De Rinaldis, N. Raventos, R. Sahyoun, & P. Guidetti Large, Large scale-variability in otolith microstructure and microchemistry: The case study of Diplodus sargus sargus (Pisces: Sparidae) in the Mediterranean Sea, *Italian Journal of Zoology*, 78, **2011**, 182
[3] Melinda M. Ranaldi, Marthe M. Gagnon, Trace Metal Incorporation in Otoliths of Black Bream (Acanthopagrus butcheri Munro), an Indicator of Exposure to Metal Contamination, *Water Air Soil Pollut*, 94, **2008**, 31

ANA-PO-38 An innovative derivatisation GC/MS procedure for the identification of proteins in the Paint Microsample

A. Pennetta, E. Margapoti*, G. E. De Benedetto*, D. Fico

Laboratory of Chemical Analyses for Cultural Heritage, Department of Fine Arts and History, University of Salento, 73100, Lecce, Italy
* Rete A.I.Te.C.H.,Via per Monteroni (c/o IBAM-CNR), 73100, Lecce, Italy
antonio.pennetta@unisalento.it

The complex chemical composition of paint layers in artworks may be related to many factors: the technique followed by the artist, the effect of aging and the environment, and the effect of past conservation practices [1]. The chemical characterization of materials used in the creation and restoration of a painting is extremely useful for surveying historical events and for gaining a better knowledge of the artistic heritage.
For determining proteinaceous materials it is necessary a multistep chemical pretreatment of the samples based on the ammonia extraction of proteins and polysaccharide materials, in order to separate them from lipid and resinous materials [2]. The proteinaceous fraction is analysed by GC-MS after hydrolysis and derivatisation of the free amino acids. A new derivatisation GC/MS procedure for the identification of proteins in the same microsample from painted works of art has been optimized. The amino acid fraction is derivatized using as solvent anhydrous dimethylformamide (DMF) instead of pyridine. More polar solvent such as pyridine is used more often because they tend to facilitate the reaction. Pyridine is an excellent solvent for TMS reactions. Although some regard pyridine as a silylation catalyst, there are many instances in which silylation reactions actually are slower in pyridine than other solvents. In addition, pyridine also may have other undesirable effects such as the promotion of secondary products and other chromatographic anomalies. DMF is used extensively, especially for large molecules. Using DMF we can limit the formation of by-products and improve the resolution of hydrophilic amino acids such as proline and hydroxyproline. The method was tested on reference materials for the identification of proteinaceous binders (egg, collagen, casein) on the basis of the quantitative determination of the amino acid profile.

[1] U.Baldini, Teoria del restauro e Unità di metodologia; *Nardini Editore*, Firenze, **1979-1981**.
[2] A.Liuveras, N. Bonaduce, A. Andreotti, M. Colombini, GC-MS Analytical procedure for the characterization of glycerolipids, Natural Waxes, Terpenoid resins, Proteinaceous and Polysaccharide Materials in the Same Paint Microsample Avoiding Interferences fron Inorganic Media*; Analytical Chemestry*, V.82., **2010**.

ANA-PO-39 A study on the curing and ageing of alkyd paints layers

L. Ghezzi, J. La Nasa, S. Orsini, F. di Girolamo, F. Modugno, C. Duce, I. Bonaduce, M. Tiné, M.P. Colombini

Dipartimento di Chimica e Chimica Industriale, Università di Pisa.

The range of modern oil-based paint media nowadays used by artists has expanded far beyond the traditional drying oils. Alkyd paints were introduced in the 1940s as alternative for traditional drying oils, and they became immediately widespread given their ability to dry fast. Chemically, alkyds are oil-modified polyesters manufactured from polyols (tipically 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 investigated the chemical transformations occurring in alkyd paint layers during curing and ageing by a multi-analytical approach entailing: direct exposure-mass spectrometry (DE-MS), gas chromatography /mass spectroscopy (GC/MS), analytical pyrolysis coupled with gas chromatography /mass spectroscopy (Py-GC/MS), Differential Scanning Calorimetry (DSC) and thermogravimetric analysis (TGA). Aim of the research was to achieve a complete characterization of the paints, to study autoxidation during curing and ageing, and to investigate the effect of the pigment.

Two kinds of alkyd paints were studied: Ferrario and Griffin, Windsor & Newton, containing both inert and metallic pigments. The paints were used to prepare reference layers that were naturally and artificially aged. Preparation of some of the paint layers was in collaboration with the IFAC CNR group (Florence).

In particular, GC/MS analysis after hydrolysis and silylation permitted to identify the fatty acid profile and the aromatic fraction of the paint, and to study molecular changes associated to curing and ageing as oxidation of double bonds.

TGA permitted to investigate the thermal stability and the thermo-oxidative behavior of alkyd paints. In particular it allowed us to achieve information on the interactions taking place between inorganic and organic species within the paint film, to investigate the occurrence of cross-linking, oxidation and hydrolysis phenomena in alkyd paints during ageing .

DSC has been used to study the oxidative polymerisation of alkyd coatings and to quantify the peroxides formed during oxidative drying. Peroxidation of fatty acid chains is the primary step in oxidative process and can used to control their drying extent.

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

ANA-PO-40 Analysis of perfluorinated acids by online SPE-LC/MS/MS

Alessandro Bacaloni,[a] Carlo Crescenzi,[b] Maria Anna Euterpio,[b] Anna Laura Capriotti,[a] Roberto Samperi,[a] Aldo Laganà[a]

[a] Dipartimento di Chimica della Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185, Rome, Italy
[b] Dipartimento di Scienze Farmaceutiche e Biomediche Università di Salerno, Via ponte don Melillo, 84084 Fisciano (SA), Italy
alessandro.bacaloni@uniroma1.it

Perfluorinated alkyl sulfonic acids (PFSAs) and perfluorinated carboxylic acids (PFCAs) are two classes of compounds widely used for industrial and household applications. They are presents in hydrorepellant coatings, stain repellents, fire-fighting foams, paints, cleaning products (especially for metal surfaces and carpets) etc. Because their resistance to degradation in the environment and their bioaccumulation properties, PFSAs and PfCAs are considered emerging pollutants and potential endocrine disrupters. During the last decades PFSAs and PFCAs spread in any compartment of the environment and nowadays can be considered ubiquitary. Due to the concern over possible effect on human health, in 2009, Stockholm Convention on Persistent Organic Pollutants suggested to impose restrictions to the use perfluorooctane sulfonic acid (PFOS) [1] and, US-EPA [2] intends to propose actions in 2012. In order to carefully evaluate the risks arising from their presence efficient analytical procedures are required. The aim of the study was to evaluate the feasibility of improving the performances of conventional reversed phase using graphic materials. The use of porous graphitic carbon (PGC) as stationary phase relevantly increase breakthrough volumes generating reproducible chromatographic conditions compatible with robust and sensitive ESI detection. Under described conditions LOD are ranged. In this study, the advantages of using online SPE was also deeply investigated. The method significantly improves previous methodology in terms of organic solvent use reduction, shorter analysis time, reduced sample manipulation and risks of contaminations. Moreover, online methods can be easily automatised.

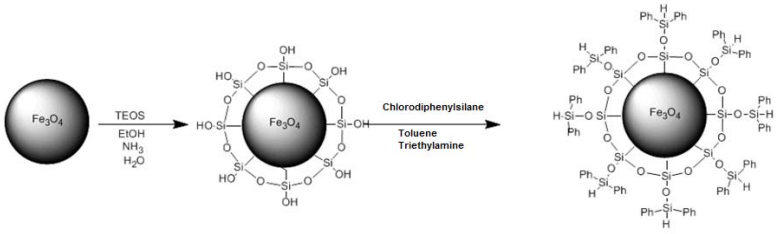
[1] SC News Release 2009/04, Geneva, Stockholm Convention Secretariat, 8 May 2008.
[2] U.S. Environmental Protection Agency 12/30/2009, Long-Chain Perfluorinated Chemicals (PFCs) Action Plan.

ANA-PO-41 Magnetic solid-phase extraction based on diphenyl functionalization of Fe_3O_4 magnetic nanoparticles for the determination of polycyclic aromatic hydrocarbons in urine samples

F.Bianchi[1], V. Chiesi[2], F. Casoli[2], L. Nasi[2], M. Careri[1], A. Mangia[1]

[1]Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Università degli Studi di Parma, Parco Area delle Scienze 17/A 43124 Parma
[2]IMEM – CNR, Parco Area delle Scienze 37/A, 43124 Parma, Italy
E-mail: federica.bianchi@unipr.it

Superparamagnetic Fe_3O_4 diphenyl nanoparticles (Scheme 1) were prepared according to a solvothermal procedure and characterized by X-ray diffraction, infrared spectroscopy and transmission electron microscopy.



Scheme 1

The magnetic phases present in the nanoparticles samples were analyzed by thermomagnetic analysis and the magnetic properties of the samples were studied by vibrating sample magnetometry. The resulting nanoparticles having an average diameter of 200 nm and characterized by a Ms value of 20 emu/g were then used as solid-phase extraction sorbents to extract polycyclic aromatic hydrocarbons from urine samples. Method validation proved the feasibility of the developed beads for the quantitation of the investigated compounds by gas chromatography-mass spectrometry at trace levels, limits of quantitation being in the ng/l range [1]. CV was always lower than 15%, which points to a good precision of the method. Finally, the superior extraction performance of the synthesized nanoparticles with respect to commercially available beads was proved.

[1] Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, May, 2001

ANA-PO-42 HPLC/MS^2 to detect and analyse TATP and HMTD in swabs.

Sabino Napoletano[a], Camilla Montesano[a], Francesco Saverio Romolo[b], Manuel Sergi[c], Luigi Cassioli[d], Silvana Grossi[d], Dario Compagnone[c], Roberta Curini[a], Giuseppe D'Ascenzo[a]

[a] Department of Chemistry, SAPIENZA University of Rome, P.le A. Moro 5, 00185 Rome, Italy
[b] Department of Legal Medicine, SAPIENZA University of Roma, Viale Regina Elena 336, 00161 Rome, Italy
[c] Department of Food Science, University of Teramo, Via Carlo Lerici 1, 64023 Mosciano Stazione (TE), Italy
[d] Italian Air Force, Flight Test Center, Armament Division, Aeroporto M. De Bernardi, Pratica di Mare, 00040 Pomezia (RM), Italy
sabino.napoletano@uniroma1.it

Peroxide-based explosives such as triacetone triperoxide (TATP) and hexamethylene triperoxide diamine (HMTD) are not difficult to synthesize and synthesis can be performed starting from readily available basic chemicals: this led to increasing use of TATP and HMTD by terrorists [1]. Whenever there is the need of collecting traces of explosives, both post blast and post transfer, surface sampling plays a critical role, especially because only reasonably small objects can be sent to a laboratory to be analyzed. Traces can also be searched on hands of suspect, where they can disappear faster than from objects.

In this work TATP and HMTD were synthesized and spiked solutions or aliquots of a few milligrams of explosive compounds were then spread on different surfaces (e.g. floors, tables) or used in handling tests. Three different swabbing systems were used: dry paper swabs, cotton swabs wetted with propan-2-ol and a commercial swab, pre-wetted with propan-2-ol and water (7:3). A simple solvent extraction procedure from swab was developed with quantitative recoveries. Paper and commercial swabs were used also to sample a metal plate, where a small charge of about 4 g of TATP was detonated. Both ESI and APCI ion sources were exploited for better ionization and fragmentation condition of analytes. All the three swabbing systems gave some positive results. The developed method was validated and showed its suitability to be used in real cases, allowing TATP detection in several simulations. Confirmation by HPLC/MS^2 was essential to give proper forensic identification of analytes and low limits of detection were reached.

[1] R. Schulte-Ladbeck et. al., *Anal.Bioanal.Chem.*, 386, **2006**, 559

ANA-PO-43 Application of 3 µm Particle-Based Amylose-Derived Chiral Stationary Phases for the Enantioseparation and Absolute Configuration Determination of Potential Histone Deacetylase Inhibitors

Rosella Ferretti[a], Antonello Mai[b], Sergio Menta[a], Bruno Gallinella[a], Leo Zanitti[a], Sergio Valente[b], Roberto Cirilli[a]

[a] Istituto Superiore di Sanità, Dipartimento del Farmaco, Viale Regina Elena 299, 00161, Rome, Italy.

[b] Istituto Pasteur - Fondazione Cenci Bolognetti, Dipartimento di Chimica e Tecnologie del Farmaco, Università degli Studi di Roma "La Sapienza" P.le A. Moro 5, 00185 Rome, Italy.

rcirilli@iss.it

Enantioselective analysis covers a significant area of analytical chemistry and it has been gaining a growing interest within a wide variety of fields dealing with drugs, flavours, fragrances, natural products and so on. The importance of this topic in view of both basic research and industrial application has given a strong impulse to the design of new and efficient chiral stationary phases (CSPs) to be used in HPLC. Currently more than 100 CSPs for HPLC are available on the market. Among them the benzoates and arylcarbamates of cellulose and amylose have been successfully employed to resolve a broad range of chiral compounds on analytical and preparative scale. Only recently (2008) amylose-derived CSPs based upon 3 µm silica (Chiralpak IA-3 and Chiralpak AD-3) were developed for applications with organic and aqueous mobile phases.

The chiral selector of both CSPs is the same, the tris(3,5-dimethylphenylcarbamate) of amylose, but it is physically coated in the case of Chiralpak AD-3 and immobilised in the Chiralpak IA-3. In this work, we report on the difference in versatility and performance of the two 3 µm particle-based CSPs towards four racemic cinnamyl 2-aminoanilides (Figure 1), endowed with HDAC inhibitory activity. The 3 µm CSPs were explored to determine if they could provide a rapid resolution of enantiomers in analytical conditions in presence of alcoholic eluents such as pure 2-propanol. The second part of work was devoted to the optimization of the mg-scale enantioseparations using an analytical 100 x 4.6 mm i.d. column. Finally, the isolated enantiomers were submitted to chiroptical analysis and their absolute configuration was established by a combined strategy based on chemical correlation/circular dichroism methods.

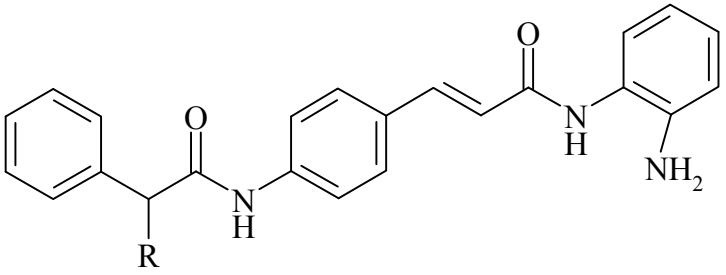


Figure 1.

ANA-PO-44 Extending the applicability of pressurized hot water extraction to compounds exhibiting limited water solubility by pH control: Curcumin from the Turmeric rhizome

Carlo Crescenzi,[a] Anna Laura Capriotti,[b] Chiara Cavaliere[b], Maria Anna Euterpio,[a] Roberto Samperi,[b] Aldo Laganà[b]

[a] Dipartimento di Scienze Farmaceutiche e Biomediche Università di Salerno, Via ponte don Melillo, 84084 Fisciano (SA), Italy
[b] Dipartimento di Chimica della Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185, Rome, Italy
carlo.crescenzi@unisa.it

Pressurized hot water extraction (PHWE; also known as subcritical water extraction, SWE) is commonly considered to be an environmentally-friendly extraction technique that could potentially replace traditional methods that use organic solvents [1]. Unfortunately, the applicability of this technique is often limited by the very low water solubility of the target compounds, even at high temperatures [2].

In this paper, the scope for broadening the applicability of PHWE by adjusting the pH of the water used in the extraction is demonstrated in the extraction of Curcumin (which exhibit very limited water solubility) from untreated turmeric (*Curcuma longa*, L.) rhizomes. Although poor extraction yields were obtained even at high temperatures when using degassed water or neutral phosphate buffer as the extraction medium, yields exceeding those obtained by Soxhlet extraction were achieved using acidic pH buffers. Optimized conditions for the extraction of Curcumin from turmeric by PHWE were identified, and the influence of the temperature, pH, and buffer concentration on the extraction yield were investigated in detail by means of a series of designed experiments. The relationships between these variables were subjected to statistical analysis using response surface methodology.

[1] T. Hyötyläinen, Critical evaluation of sample pretreatment techniques, *Anal. Bioanal. Chem*., 394, **2009**, 743.
[2] S.B. Hawthorne, Y. Yang, and D.J. Miller, Extraction of Organic Pollutants from Environmental Solids with Sub- and Supercritical Water, *Anal. Chem*., 66, **1994**, 2912.

ANA-PO-45 Capillary electrophoresis of *Escherichia coli*: a first attempt

Fabiana Crispo,[a] Lucia Bruno,[a] Rosanna Ciriello,[a] Angela Ostuni,[a] Faustino Bisaccia,[a] Antonio Guerrieri [a]

[a]Dipartimento di Chimica, Università degli Studi della Basilicata, via dell'Ateneo Lucano 10, 85100 Potenza, Italy
fabiana.crispo@unibas.it

In the last few years, capillary electrophoresis have been used to characterize and separate microorganisms on the bases of their electrophoretic mobility [1]. Indeed microorganisms have an external structure, the "cell wall", with a characteristic molecular composition which distinguishes microbial species and strains from each other (yeasts, bacteria, viruses). More importantly, the cell wall contains several ionizable groups that in particular conditions give rice to a superficial charge causing them to migrate with a typical migration time under an applied electric field [2,3]. Our previous works on backer's yeast [4] have demonstrated that the characteristic electropherogram of this species shows two peaks ascribed to neutral and negative charged cells. Relevant microscopic studies have further pointed out that the electrophoretic profile also reflects the dimensional distribution of cells in the analyzed sample.
In the following work, our interest has been focused on the electrophoretic behavior of *Escherichia coli*, a microorganism of significant interest for its role in several infectious diseases and its importance in biotechnological industries. As a first attempt, it was necessary to optimize the electrophoretic conditions for the identification and efficient separation of this microorganism by capillary electrophoresis; accordingly, the effects of the running buffer, pH, the separation voltage and the microbial aggregation on the electrophoretic profile of *Escherichia coli* were studied. Once typical electropherogram of that bacteria was identified, a sample containing *Saccharomyces cerevisiae* and *Escherichia coli* was analyzed using capillary electrophoresis and the experimental results have demonstrated the power of the technique in detecting the bacterial contamination of fungal sample.
Thus capillary electrophoresis is able to replace traditional method of microbial identification because it permits rapid, easy and highly sensitive microbial analysis and diagnoses at low costs on several biological samples.

[1] E.Kłodzińska, B.Buszewski, *Analytical Chemistry*, 81, **2009**, 8-15.
[2] A.T.Poortinga, R.Bos, W.Norde, H.J.Busscher, *Surface Science Report*. 47, **2002**, 1-32.
[3] S.P.Radko, A.Chrambach, *Electrophoresis*, *23*, **2002**, 1957-1972.
[4] F.Crispo, A.Capece, R.Ciriello, P.Romano, A.Guerrieri, paper under submission.

ANA-PO-46 Characterization of non-volatile polyphenol compounds in coniferae by liquid chromatography-mass spectrometry

Natalia B. Czapczyńska,[a] Anna Laura Capriotti,[b] Chiara Cavaliere,[b] Carlo Crescenzi,[c] Andrzej L. Dawidowicz,[a] Patrizia Foglia,[b] Roberto Samperi,[b] Aldo Laganà[b]

[a] Department of Chromatographic Methods, Maria Curie - Sklodowska University, pl. Maria Curie - Sklodowska 3, 20-031 Lublin, Poland

[b] Dipartimento di Chimica della Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185, Rome, Italy

[c] Dipartimento di Scienze Farmaceutiche e Biomediche Università di Salerno, Via ponte don Melillo, 84084 Fisciano (SA), Italy

natalia.cz@wp.pl

Vascular plants synthesize a large number of diverse biochemical compounds with specialized functions. It has been estimated that up to 200,000 different metabolites occur in the plant kingdom, and each species may contain its own chemotypic expression pattern [1]. Secondary metabolites are present in all higher plants, usually in a high structural diversity. As a rule, a single group of phytochemicals dominates within a given taxon. Plant phenolics are one of the most important groups of secondary metabolites in plants and approximately 8000 of them are known. From a structural point of view, phenolic compounds include a wide range of substances: simple phenols, phenolic acids, phenylpropanoids, coumarins, quinones, flavonoids, tannins, and other miscellaneous phenols [2].

A liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS) method employing a time-of-flight (TOF) analyzer with LockSpray source for continuous accurate mass measurements was developed for evaluation of phenolic content in some coniferae, i.e. pine (*Pinus Pinea*), cypress (*Cupressus sempervirens* L.) and thuja (*Thuja* L.).

Matrix Solid Phase Dispersion (MSPD) using C18 as dispersing material and methanol as eluent was applied to extract the analytes from matrices. The fragmentation patterns of phenolic compounds were obtained using both positive and negative ion mode, and accurate mass data were used for identification of compounds. In all the three plant extracts, various phenolic compounds were identified, most of them belonging to flavonoid group. Cypressus extract showed the richest chromatographic profile compared to pine and thuja extracts.

Recovery and quantitative estimation were performed by using biochanin A (not present as endogenous compound in the extracts) as internal standard and other selected analytes as references of the various compound typologies (aglycones, monoglycosilated, and diglycosilated).

[1] J.J.B. Keurentjes, J. Fu, C.H.R. de Vos, A. Lommen, R.D. Hall, R.J. Bino, L.H.W. van der Plas, R.C. Jansen, D. Vreugdenhil, and M. Koornneef, *Nature Genetics, 38*, **2006**, 842

[2]R. Robbins, *J. Agric. Food Chem., 51,* **2003**, 2866

ANA-PO-47 Single column Ag^+-HPLC analysis of CLA isomers

De Michele A., Blasi F., Cossignani L.

Dipartimento di Scienze Economico-Estimative e degli Alimenti, Sezione di Chimica Bromatologica, Biochimica, Fisiologia e Nutrizione, Università degli Studi di Perugia, Via San Costanzo 1, 06126 Perugia, Italy
E-mail: adm978@libero.it

It is known that conjugated linoleic acid (CLA) isomers possess many important biological properties, including anticarcinogenic, antidiabetogenic and antiadipogenic effects [1]. The most CLA found in human tissues is from dietary origin (meat and dairy products), because endogenous synthesis is very limited [2]. Therefore many nutritional complements containing CLA, as free fatty acids, alkyl ester or triacylglycerol mixtures, are now available. Since CLA biological effects are isomer-specific, it is very important to have reliable and precise techniques for identification and quantification of CLA isomers in food and nutritional supplements. A combination of high resolution gas chromatography and multi-column silver-ion high performance liquid chromatography (Ag^+-HPLC) was found to be necessary to resolve all CLA isomers [3].

In this research a CLA standard mixture, containing four *c,t*/*t,c* positional isomers (*c,t*-11,13, *t,c*-10,12, *c,t*-9,11 and *t,c*-8,10), was derivatized using different length chain alcohols; the obtained compounds were analyzed by Ag^+-HPLC coupled with UV detection system, set at 232 nm. The analytical technique was optimized by using a single Ag^+ column to separate different alkyl esters of CLA isomers. The use of a single column allowed to obtain the separation of CLA isomers in less time and with lower cost with respect to multi-column Ag^+-HPLC. Good reproducibility of CLA isomer retention times was obtained using daily prepared mobile phase and a column oven. The separation degree of the four CLA isomers increased with the alkyl chain length. The best resolutions were observed for hexyl ester derivatives.

The optimized analytical technique could represent a useful tool to evaluate the quali- and quantitative profile of CLA isomers in nutritional supplements so to provide accurate information for the consumers.

[1] M.W.Pariza, Y.Park, and M.E.Cook, *Prog.LipidRes.*, *40*, **2001**, 283.
[2] I.Salminen, M.Mutanen, M.Jauhiainen, and A.Aro, *9*,**1998**, 93.
[3] N.Sehat, J.K.G.Kramer, M.M.Mossoba, M.P.Yurawecz, J.A.G.Roach, K.Eulitz, K.M.Morehouse, and Y.Ku, *Lipids, 33*, **1998**, 963.

ANA-PO-48 Fluorinated stationary phases for alternative selectivity. Separation of compounds of biological and biomedical relevance

Anna Laura Capriotti,[a] Alberto Cavazzini,[b] Alessia Ciogli,[c] Patrizia Foglia,[a] Francesco Gasparrini,[c] Aldo Laganà,[a] Nicola Marchetti,[b] Luisa Pasti,[b] Claudio Villani[c]

[a] Dipartimento di Chimica dell'Università di Roma "La Sapienza", Piazzale Aldo Moro 5, 00185, Roma, Italy

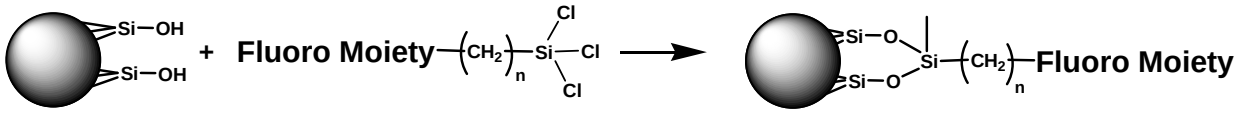
[b] Dipartimento di Chimica dell'Università di Ferrara, via L. Borsari 46, 44121 Ferrara, Italy

[c] Dipartimento di Chimica e Tecnologie del Farmaco dell'Università di Roma "La Sapienza, Piazzale Aldo Moro 5, 00185, Roma, Italy

patrizia.foglia@uniroma1.it

In recent years, growing interest has been posed in the use of fluorinated stationary phases (FSPs) in liquid chromatography, because of their orthogonality respect to traditional alkyl phases. In fact fluorinated phases are characterized by alternative retention mechanisms, in particular show more polar interactions compared to traditional alkyl phases, offering a different selectivity [1-3].

We prepared new FSPs, containing polyfluorinated aryl or alkyl fragments bound to silica with different particle sizes (1.7-5.0 micron) and pore size of 100 or 300 Å. In order to improve the stationary phase chemical stability, the matrix synthesis is based on the employment of functional silanes, where the polyfluorinated fragment is located far from silicon atom by an non-fluorinated alkyl spacer according to the following scheme:



These new phases, packed in columns of different geometries (spanning from standard to nano-) were compared to conventional alkyl-bonded phases (C18, C8, phenyl-) analyzing the loading density and the nature of the stationary phases in terms of hydrophobicity, selectivity for neutral (CH_2 selectivity) and polar solutes.

Moreover, the new FSPs have been employed for the HPLC-MS analysis of halogenated compounds and also for the separation of biological and biomedical relevant peptides whose selective retention could be influenced by the stationary phase fluorination.

[1] D.P. Curran, *Synlett, 9,* **2001**, 1488

[2] T. Hayama et al., *Rapid Commun. Mass Spectrom.,24,* **2010**, 2868

[3] H. Glatz et al., *Chromatographia, 59,* **2004**, 567

ANA-PO-49 Purification of bistrifluorosulfonimide (NTf_2) based Ionic Liquids for electrochemical and photochemical purposes

Daniele Merli, Paola Petracca, Stefano Protti, Maurizio Fagnoni, Antonella Profumo

Dipartimento di Chimica dell'Università di Pavia, Via Taramelli 12, 27100, Pavia, Italy
daniele.merli@unipv.it

Ionic liquids (ILs) are peculiar compounds with several smart properties, they are liquid at room temperature, with high thermal and chemical stabilities and they can be synthesized in several different combinations to modulate their properties in view of their use. [1]
Their synthesis is normally easy and fast, but the products often contain great amounts of water and other impurities that could impart to them a yellowish color and poor electrochemical properties.[2]
In the present work a purification protocol was developed that can lead to suitable ILs to be used as supporting electrolytes for electrochemical applications and/or as solvents for photochemical reactions.
Water immiscible ILs are preliminary investigated namely $[Py_{1,4}]^+[NTf_2]^-$, $[Py_{1,102}]^+[NTf_2]^-$, $[bmim]^+[NTf_2]^-$.The purification protocol of the IL involves a washing step with water in a liquid/liquid extractor, treatment with activated carbon and C-18 resin disk, and a drying step by using alumina or calcium hydride.
The effect of each purification step was evaluated by fluorescence spectroscopy, UV-Vis, NMR, IR, GC-MS and cyclic voltammetry.
It was shown that the purification leads to an improvement of the electrochemical stability and to a better reversibility of the redox probe ferrocene.
A well-known photochemical reaction, viz the addition of a photogenerated phenyl cation onto benzene [3], was performed in unpurified and purified $[Py_{1,102}]^+[NTf_2]^-$, showing the effectiveness of the purification protocol and the chemoselectivity imparted to the reaction by the IL itself.

[1] K.N.Marsh, J.A. Boxall and R. Lichtenthaler *Fluid Phase Equilibria* **2004**, *4*, 107.
[2] P. Nockemann, K. Binnemans and K. Driesen *Chem. Eng. Res. Des*., **2008**, *86,* 775.
[3] V. Dichiarante,C. Betti, M. Fagnoni, A. Maia, D. Landini and A. Albini *Chem. Eur. J.*, *13*, **2007**, 183.

ANA-PO-50 An efficient and reliable DoE optimized SPME-GC-MS/MS method for determination of hydrazine in drinking water

Emanuela Gionfriddo, Attilio Naccarato, Giovanni Sindona, Antonio Tagarelli

Dipartimento di Chimica, Università della Calabria, via P. Bucci Cubo 12/C 87036 Arcavacata di Rende (CS), Italy
egionfriddo@unical.it

Hydrazine is a genotoxic and neurotoxic compound, classified as probable human carcinogen by the U.S Environmental Protection Agency. Hydrazine drinking water contamination can originate from military and industrial wastes, wastewater treatment plant (WWTP) effluents, and possible formation in drinking water disinfection [1]. Various papers describes hydrazine determination by gas chromatography by different extraction and derivatization techniques [2],[3]. The purpose of this work was the development of a method for the determination of hydrazine in drinking water by solid-phase microextraction coupled with gas chromatography-tandem mass spectrometry (SPME-GC-MS/MS). Derivatization reaction was carried out using chloroformates in accordance with the procedure proposed by Hušek [4]. Derivatization parameters are investigated by the multivariate approach of "Experimental Design" in order to find the best experimental condition that lead to formation of the di-derivatizated compound only. For this purpose are tested three different alkyl chloroformate (ethyl, propyl and isobutyl). The performances of five fibers (both in DI and HS mode) and three chloroformates were surveyed by mean of a 5×3 multi-factor categorical design. From this study the fiber and the chloroformate that gave best results were selected for experimental design optimization in order to improve the extraction process. The variables affecting thermodynamic aspects of SPME such as desorbtion temperature and NaCl concentration will be optimized by a central composite design (CCD). Sample extraction time and agitation, which are variables that affecting kinetic aspect, will be optimized by the univariate method.

[1] U.S. Environmental Protection Agency (EPA). *Fed. Regist.* **2006**, *71* (2),(40 CFR Parts 9, 141, and 142).
[2] M. Y. Khuhawar and L. A. Zardari, *Analytical Science,* **2008**, 24, 1493.
[3] W.E. Davis, Y. Li, *Anal. Chem.,* **2008**, 80, 5449.
[4] P. Hušek, *J. Chromatogr.*, **1991**, 289, 552.

ANA-PO-51 Electrochemical Properties and Analytical Application of Bio-Nano-Electrodes

Walter Vastarella, Livia Della Seta, Maria Rita Montereali, Carlo Cremisini
ENEA, C.R.E. Casaccia, Via Anguillarese 301, 00123, Santa Maria di Galeria, Rome, Italy
walter.vastarella@enea.it

Gold electrodes are mainly used because of their window of working potential and the possibility to easily functionalize their surface by self assembled monolayers (SAMs) or electrochemically deposited multilayers (EDM) through thio- or amino-coordinate derivatives [1]. By using nanoporous membranes as templates for Nano Electrode Ensembles (NEE), it is possible to obtain an assembly of gold ultra-microelectrodes confined in areas to a few mm^2 , which can be either used such as or as platform for further immobilization of biomolecules [2]. Gold NEE were synthesized within porous polycarbonate membranes and coupled with a screen printed substrate to give a disposable and versatile electrochemical system for biosensing and bio analytical applications. Scanning Electron (SEM) and Scanning Probe Microscopy (SPM) techniques have been used to characterize the gold nanostructures morphology. Efficiency and sensitivity of the electrodes so obtained were tested using glucose-oxidase immobilized on the nanosized surface. Electrodes responses to hydrogen peroxide and glucose were collected and compared to other glucose-oxidase macro-electrodes [3]. Different enzymes were immobilized on the nano-sensor surface and different immobilization techniques have also been taken into account. Novel nanowire depositions (using Ni, Pt, Ag) in template nanosystems have been recently experimented for several bioanalytical applications, e.g. in detection of phenolic compounds, choline and biogenic amines.

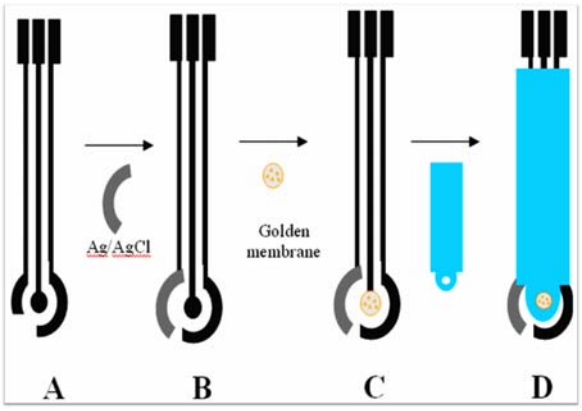


Figure 1- Schematic sequence of NEE preparation on screen printed substrate (SPS):
A) carbon graphite tracks and contact pads obtained by screen printing; B) Ag/AgCl paste deposition (reference electrode); C) placement of the NEE based membrane on the working electrode; D) dielectric paste deposition

[1] J Maly, M Ilie, V Foglietti, E Cianci, A Minotti, L Nardi, A Masci, W Vastarella, R Pilloton, *Sensors and Actuators* B, **2005**; 111/112: 317-322
[2] VP Menon, CR Martin, *Anal. Chem.*, **1995**; 67(13): 1920-1928
[3] W Vastarella, L Della Seta, A Masci, J Maly, M De Leo, LM Moretto, R Pilloton, *Int. J. Environ. Anal. Chem.*, **2007**; 87(10-11): 701-714