

# XXIII CONGRESSO NAZIONALE DELLA DIVISIONE DI CHIMICA ANALITICA DELLA SOCIETÀ CHIMICA ITALIANA

ISOLA D'ELBA, 16-20 SETTEMBRE 2012

## ATTI (Abstract Book)



*Divisione di Chimica Analitica*  
SOCIETÀ CHIMICA ITALIANA



*Università degli Studi di Firenze*

# **Analitica 2012**

ISOLA D'ELBA

16-20 Settembre, 2012

XXIII congresso nazionale della divisione di chimica analitica della S.C.I.

*Con il patrocinio dell'Università degli Studi di Firenze*

# ATTI (*ABSTRACT BOOK*)

e

Programma finale



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a cura di Sergio Pinzauti

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*La Chimica Analitica per la vita e per l'ambiente*

## Programma

### Giorno 1 • Domenica, 16 Settembre 2012

14:30-18:00 Registrazione dei partecipanti

### Sessione Plenaria 1

SALA MARIA LUISA (SALA A)

18:00-18:30 Apertura del Congresso (*G. Arena, Presidente Div. Chim. Anal. S.C.I., A. Tesi, Rettore Università di Firenze*)

PRESIEDE: G. ARENA (CATANIA)

18:30-19:10 **Conferenza Plenaria 1**

WHAT ARE THE ANALYTICAL NEEDS FOR THE REACH  
REGULATION?

*Ph. Garrigues, University of Bordeaux1, France*

19:10-20:00 *Cocktail di benvenuto*

20:00 *Cena di benvenuto con intrattenimento musicale*

### Giorno 2 • Lunedì, 17 Settembre 2012

### Sessione Plenaria 2

SALA A

PRESIEDE: A. RODA (BOLOGNA)

09:00-09:40 **Conferenza Plenaria 2**

RECENT DEVELOPMENTS IN ENANTIOSELECTIVE  
ANALYSIS

*B. Chankvetadze, University of Tbilisi, Georgia*

### Sessione Parallela 1. Sensori e Elettroanalisi

SALA A

PRESIEDE: L. TORSI (BARI)

09:50-10:20 **Keynote Lecture 1**

SENSING SYSTEMS: STATE-OF-THE-ART AND  
PERSPECTIVES

*R. Seeber, University of Modena and Reggio Emilia*

### Oral Communications 1-5

- 10:20-10:40 LIGHT-ACTIVATED ELECTROSYNTHESIS OF MICROSTRUCTURED MOLECULARLY IMPRINTED POLYMERS FOR SENSING APPLICATIONS  
*E. Mazzotta<sup>1</sup>, C. Malitesta<sup>1</sup>, S. Surdo<sup>2</sup>, G. Barillaro<sup>2</sup> (<sup>1</sup>Salento University, <sup>2</sup>University of Pisa)*
- 10:40-11:00 DEVELOPMENT OF SENSORS BASED ON SCREEN PRINTED ELECTRODES MODIFIED WITH CARBON BLACK AND GOLD NANOPARTICLES NANO-COMPOSITE  
*F. Arduini<sup>1</sup>, C. Zanardi<sup>2</sup>, S. Cinti<sup>1</sup>, N. Alaimo<sup>1</sup>, F. Terzi<sup>2</sup>, R. Seeber<sup>2</sup>, D. Moscone<sup>1</sup>, G. Palleschi<sup>1</sup> (<sup>1</sup>University of Rome Tor Vergata, <sup>2</sup>University of Modena and Reggio Emilia)*
- 11:00-11:20 *Coffee Break*
- 11:20-11:40 AN ELECTROCHEMICAL GAS SENSOR BASED ON PAPER SUPPORTED ROOM TEMPERATURE IONIC LIQUIDS INTENDED FOR THE ANALYSIS OF ACID SPECIES  
*N. Dossi, R. Toniolo, A. Pizzariello, G. Bontempelli (University of Udine)*
- 11:40-12:00 AFFINITY ELECTROCHEMICAL BIOSENSORS BASED ON NANO-ELECTRODE ENSEMBLES  
*M. Silvestrini, L.M. Moretto, P. Ugo (Ca' Foscari University of Venice)*
- 12:00-12:20 RATIONALIZATION OF THE SIGNAL DRIFT NATURE OF OXYGEN OPTICAL SENSORS AND ITS EXPERIMENTAL CHECK WITH A LIGHT INTENSITY DETECTION BASED SENSOR  
*D. Badocco, A. Mondin, P. Pastore (University of Padua)*

## Sessione Parallela 2. Scienza delle Separazioni

SALA BONAPARTE (SALA B)

PRESIEDE: F. DONDI (FERRARA)

09:50-10:20 **Keynote Lecture 2**

CAPILLARY ELECTROPHORESIS AND HPLC OF BIOMOLECULES: FUNDAMENTAL AND PRACTICAL ASPECTS RELATED TO THE OPTIMIZATION OF THEIR SEPARATION PERFORMANCE

*D. Corradini, A. De Rossi, I. Nicoletti, CNR, Rome*

### Oral Communications 6-10

- 10:20-10:40 DETERMINATION OF PCB IN SOIL SAMPLES USING MICROWAVE ASSISTED EXTRACTION FOLLOWED BY GAS CHROMATOGRAPHY  
*M.C. Bruzzoniti<sup>1</sup>, R. Maina<sup>2</sup>, V. Tumiatti<sup>2</sup>, C. Sarzanini<sup>1</sup>, L. Rivoira<sup>1</sup>, R.M. De Carlo<sup>1</sup> (<sup>1</sup>University of Turin, <sup>2</sup>Sea Marconi Technologies, Turin)*
- 10:40-11:00 DEVELOPMENT AND OPTIMISATION OF AN HPLC/MS/MS METHOD FOR THE DETERMINATION OF PHENOLIC ACIDS AND DERIVATIVES USING A RP-AMIDE STATIONARY PHASE  
*A. Restivo, I. Degano, E. Ribechini, M.P. Colombini (Pisa University)*

- 11:00-11:20 *Coffee Break*
- 11:20-11:40 MULTI-WALLED CARBON NANOTUBES-MODIFIED SILICA MICROSPHERES: A NEW HPLC STATIONARY PHASE  
*A. Speltini, D. Merli, D. Dondi, C. Milanese, P. Galinetto, D. Longhi, A. Profumo (University of Pavia)*
- 11:40-12:00 DEVELOPMENT OF A MATHEMATICAL MODEL FOR ONLINE MICROEXTRACTION BY PACKED SORBENT UNDER EQUILIBRIUM CONDITIONS AND ITS APPLICATION FOR POLYCYCLIC AROMATIC HYDROCARBONS DETERMINATION IN WATER BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY  
*M. Quinto<sup>1</sup>, P. Amodio<sup>2</sup>, G. Spadaccino<sup>1</sup>, D. Centonze<sup>1</sup> (<sup>1</sup>University of Foggia, <sup>2</sup>University of Bari)*
- 12:00-12:10 PHENOLIC ACIDS AND ANTIOXIDANT CAPACITY IN DURUM WHEAT AND ITS PRODUCTS  
*F. Taddei<sup>1</sup>, A. De Rossi<sup>2</sup>, D. Martini<sup>1,3</sup>, M.G. D'Egidio<sup>1</sup> (<sup>1</sup>C.R.A., Rome, <sup>2</sup>CNR, Rome, <sup>3</sup>Campus Bio-Medico, University of Rome)*
- 12:10-12:20 INSIGHTS INTO RETENTION MECHANISMS OF PERFLUOROALKYL ACIDS ON PERFLUORINATED SORBENTS. FLUOROUS AFFINITY CHROMATOGRAPHY AS A TOOL FOR ENRICHMENT AND ANALYSIS OF PERFLUORINATED EMERGING CONTAMINANTS  
*N. Marchetti<sup>1,2</sup>, A. Cavazzini<sup>2</sup>, L. Pasti<sup>2</sup>, F. Dondi<sup>2</sup>, F. Gasparrini<sup>3</sup>, A. Laganà<sup>3</sup> (<sup>1</sup>"Terra&Acqua Tech" Lab., Ferrara, <sup>2</sup>University of Ferrara, <sup>3</sup>Sapienza University of Rome)*

## Workshop 1

SALA A

PRESIEDE: D. CORRADINI (ROMA)

- 12:20-13:00 EVOLUTION OF HPLC MATERIAL TECHNOLOGY: FROM IRREGULAR TO CORE SHELL SILICA  
*A. Gheduzzi, Phenomenex*

13:00-14:30 *Lunch Break*

14:30-16:00 **Sessione Poster I**

15:40-16:00 *Coffee*

## Sessione Parallela 3. Chemiometria

SALA A

PRESIEDE: S. LANTERI (GENOVA)

- 16:00-16:30 **Keynote Lecture 3**  
NEW PERSPECTIVES IN CHEMOMETRICS  
*R. Todeschini, Università di Milano "Bicocca"*

**Oral Communications 11-15**

- 16:30-16:50 AQUAPHOTOMICS: DETERMINATION OF SALTS IN WATER BY NIR SPECTROSCOPY AND CHEMOMETRICS  
*M. Bevilacqua<sup>1</sup>, S. De Luca<sup>1</sup>, A. Gowen<sup>2,3</sup>, R. Bucci<sup>1</sup>, A.D. Magrì<sup>1</sup>, A.L. Magrì<sup>1</sup>, R. Tsenkova<sup>3</sup>, F. Marini<sup>1</sup>, (<sup>1</sup>University of Rome "La Sapienza", <sup>2</sup>Univ. College Dublin, Ireland, <sup>3</sup>Kobe Univ., Japan)*

- 16:50-17:10 MULTIVARIATE STATISTICAL OPTIMIZATION OF BIOHYDROGEN PRODUCTION FROM CRUDE GLYCEROL  
*B. Giussani<sup>1</sup>, C. Varrone<sup>2,3</sup>, F. Fiocchetti<sup>2</sup>, S. Rosa<sup>2</sup>, G. Izzo<sup>2</sup>, G. Massini<sup>2</sup>, A. Marone<sup>2</sup>, A. Signorini<sup>2</sup>, A. Wang<sup>3</sup> (<sup>1</sup>University of Insubria, <sup>2</sup>ENEA, Rome, <sup>3</sup>Harbin Inst. Tech., China)*
- 17:10-17:30 CRITICAL EVALUATION OF STRATEGIES FOR INTEGRATION OF DATA FROM DIFFERENT ANALYTICAL INSTRUMENTS: FUSION OF INFORMATION.  
*M. Casale, M.C. Casolino, S. Lanteri (University of Genoa)*
- 17:30-17:50 CHEMOMETRICS AND METABOLOMICS BASED ON LC-MS: FROM RAW DATA TO STATISTICAL MODELS  
*M. Stocchero<sup>1</sup>, F. Guzzo<sup>2</sup>, (<sup>1</sup>S-IN, Vicenza, <sup>2</sup>University of Verona)*
- 17:50-18:10 COUPLING 2D-WAVELET DECOMPOSITION AND MULTIVARIATE IMAGE ANALYSIS  
*M. Li Vigni<sup>1</sup>, M. Cocchi<sup>1</sup>, J.M. Prats Montalban<sup>2</sup>, A. Ferrer<sup>2</sup> (<sup>1</sup>University of Modena & Reggio Emilia, <sup>2</sup>Operations Res. & Quality, Valencia, Spain)*

## Sessione Parallela 4. Equilibri in Soluzione e Speciazione

SALA B

PRESEDE: P.G. DANIELE (TORINO)

16:00-16:30 **Keynote Lecture 4**

THE CHEMISTRY OF EQUILIBRIA IN SOLUTION AND RELATIVE SPECIATION STUDIES: PAST PRESENT AND FUTURE

*S. Sammartano, University of Messina*

**Oral Communications 16-20**

16:30-16:50 CHARACTERIZATION OF OXOVANADIUM(IV) COMPLEXES WITH HYDROXYLATED CARBOXYLIC LIGANDS IN AQUEOUS SOLUTION

*S. Berto, P.G. Daniele, E. Diana, E. Laurenti, E. Prenesti (University of Turin)*

16:50-17:10 INCLUSION OF ORGANIC ANIONS AND SELF-ASSEMBLY OF CALIXARENE CAPSULES IN WATER AT NEUTRAL PH

*C. Bonaccorso, C. Sgarlata, V. Zito, D. Sciotto, G. Arena (University of Catania)*

17:10-17:30 BINDING ABILITY OF GLUTATHIONE TOWARDS METAL AND ORGANOMETAL CATIONS

*G. Falcone, C. Foti, O. Giuffrè (University of Messina)*

17:30-17:50 POTENTIOMETRIC AND LASER DESORPTION MASS SPECTROMETRIC INVESTIGATION ON *trans*-HYDROXY-L-PROLINE AND Fe(III) EQUILIBRIA

*D. Aiello, E. Furia, A. Napoli, G. Sindona, A. Tagarelli (Calabria University)*

17:50-18:10 ADSORPTION OF PHOSPHOROTIOATES PESTICIDES ONTO AMORPHOUS IRON (III) PHOSPHATE

*G. De Tommaso, M. Iuliano, C. Manfredi (University of Naples)*



SALA A  
18:15 **Assemblea DCA SCI**  
20:30 *Cena e Piano Bar*

**Giorno 3 • Martedì, 18 Settembre 2012**

## **Sessione Plenaria 3**

SALA A  
PRESIEDE: A. LAGANÀ (ROMA)  
09:00-09:40 **Conferenza Plenaria 3**  
COMPANION DIAGNOSTICS FOR PERSONALIZED  
MEDICINE  
*A. Roda, University of Bologna*

## **Sessione Parallela 5. Alimenti e Nutraceutici**

SALA A  
PRESIEDE: D. COMPAGNONE (TERAMO)  
09:50-10:20 **Keynote Lecture 5**  
DEVELOPMENT OF ANALYTICAL METHODS FOR THE  
CHARACTERIZATION OF POTENTIALLY HEALTH  
BENEFITING FOODS AND FOOD INGREDIENTS  
*C. Corradini, University of Parma*

### **Oral Communications 21-25**

- 10:20-10:40 INORGANIC COMPONENTS IN HONEYS AS POTENTIAL  
INDICATORS OF BOTANICAL ORIGIN AND OF  
ANTHROPOGENIC ENVIRONMENTAL POLLUTION  
*P. Fermo<sup>1</sup>, G. Beretta<sup>1</sup>, A. Piazzalunga<sup>2</sup>, F. Biadigo<sup>1</sup>, R. Maffei  
Facino<sup>1</sup> (1University of Milan, 2Bicocca University of Milan)*
- 10:40-11:00 PRIMARY METABOLISM OF BERBERINE IN HUMAN:  
CORRELATION BETWEEN PHYSICOCHEMICAL  
PROPERTIES AND PLASMA LEVELS BY HPLC-ESI-MS/MS  
*S. Spinozzi, C. Colliva, C. Camborata, M. Roberti, C. Ianni, C.  
Calvanese, A. Lisotti, G. Mazzella, A. Roda (Bologna University)*
- 11:00-11:20 *Coffee Break*
- 11:20-11:40 COMPREHENSIVE PROFILING OF CAROTENOIDS AND FAT-  
SOLUBLE VITAMINS IN MILK FROM DIFFERENT ANIMAL  
SPECIES BY LC – DAD – MS/MS HYPHENATION  
*A. Gentili, F. Caretti, S. Bellante, R. Curini, G. D'Ascenzo  
("Sapienza" University of Rome)*
- 11:40-12:00 PRELIMINARY STUDY ON QUANTIFICATION OF  $\alpha_{S1}$ -CASEIN  
VARIANTS IN GIRGENTANA GOAT BREED BY DIRECT  
CHROMATOGRAPHIC ANALYSIS OF MILK  
*M. Montalbano, L. Tortorici, S. Mastrangelo, B. Portolano  
(University of Palermo)*
- 12:00-12:20 APPLICATION OF DIFFERENT TECHNIQUES TO DETECT  
IRRADIATED FOOD AT THE PRODUCT MARKETING STAGE  
*G. Marchesani, M. Mangiacotti, G. Siragusa, F. Floridi, A.E.  
Chiaravalle (Zooprofilattico Sperimentale Inst. of Puglia and  
Basilicata)*

## Sessione Parallela 6. Green Chemistry

SALA B

PRESIEDE: A. TAPPARO (PADOVA)

09:50-10:20 **Keynote Lecture 6**

GREEN CHEMISTRY: AN ANALYTICAL PERSPECTIVE

*L. Pasti, University of Ferrara*

### Oral Communications 26-30

10:20-10:40 THE EVOLUTION OF ENVIRONMENTAL ETHICS FOR A SUSTAINABLE WORLD

*F. Dondi<sup>1</sup>, F. Moser<sup>2</sup> (<sup>1</sup>University of Ferrara, <sup>2</sup>United Nations Environment Programme, Geneva, Switzerland)*

10:40-11:00 APPLICATIONS OF ANALYTICAL PYROLYSIS TO THE DEVELOPMENT OF FUELS AND CHEMICALS FROM BIOMASS

*D. Fabbri, C. Torri (University of Bologna)*

11:00-11:20 *Coffee Break*

11:20-11:40 MICROREACTOR TECHNOLOGY: A GREEN ANALYTICAL TOOL FOR THE STUDY AND CHARACTERIZATION OF COMPLEX REACTIONS

*A. Cavazzini<sup>1</sup>, A. Massi<sup>1</sup>, R. Greco<sup>1</sup>, N. Marchetti<sup>1,2</sup>, F. Dondi<sup>1</sup> (<sup>1</sup>University of Ferrara, <sup>2</sup>Lab. "Terra&Acqua Tech", Ferrara)*

11:40-12:00 ON THE MEASUREMENT OF THE PHOTOCATALYTIC ACTIVITY FOR THE ABATEMENT OF GASEOUS SPECIES

*C. Minero, M. Minella, V. Maurino (University of Turin)*

12:00-12:20 BINDING STUDY OF HEPARIN FROM DIFFERENT SOURCES TO ANTITHROMBIN BY AFFINITY CAPILLARY ELECTROPHORESIS

*R. Gotti<sup>1</sup>, G. Basaglia<sup>1</sup>, L. Liverani<sup>2</sup>, B. Parma<sup>2</sup> (<sup>1</sup>University of Bologna, <sup>2</sup>Opcrin S.p.a., Modena)*

## Workshop 2

SALA A

PRESIEDE: G. SCARPONI (ANCONA)

12:20-13:00 FAST GC-MS/MS ANALYSIS OF PESTICIDES (QUECHERS) USING RAPID FULL SCAN/MRM SWITCHING MODES

*F. Bruno<sup>1</sup>, S. Kitano<sup>2</sup>, H. Miyagawa<sup>2</sup>, S. Kräher and H.-U. Baier<sup>3</sup> (<sup>1</sup>Shimadzu Italia, <sup>2</sup>Shimadzu Corp., Japan, <sup>3</sup>Shimadzu Europa, Germany)*

12:40-13:00 NEW DEVELOPMENT IN SHIMADZU TANDEM MASS SPECTROMETRY AND APPLICATION IN RESEARCH OF MYCOTOXINS IN FOOD

*S. Zaza (Shimadzu Italia)*

13:00-14:30 *Lunch Break*

## Sessione Parallela 7. Miscellanea I

SALA A

PRESIEDE: S. SAMMARTANO (MESSINA)

### Oral Communications 31-36

- 14:30-14:50 STABILITY CHARACTERISATION VIA ISOTHERMAL AND SCANNING CALORIMETRY  
*M. Coletti, R. Pepi (TA Instruments, a Division of Waters ltd, Milan)*
- 14:50-15:10 CID/ETD TANDEM MASS SPECTROMETRY FOLLOWING NANO LIQUID CHROMATOGRAPHY FOR CHARACTERIZING PHOSPHOPROTEINS  
*D. Nardiello, C. Palermo, A. Natale, D. Centonze (University of Foggia)*
- 15:10-15:30 TOWARDS TOTAL AND FREE IRON(III) SENSING  
*G. Alberti, M. Zelaschi, G. Emma, R. Biesuz (University of Pavia)*
- 15:30-15:50 ADSORPTION OF SELECTED PHARMACEUTICALS BY ZEOLITES  
*E. Sarti, L. Pasti, A. Martucci, A. Cavazzini, F. Dondi (University of Ferrara)*
- 15:50-16:10 HIGH-RESOLUTION MULTY PROXY RECORD OF CLIMATIC AND ENVIRONMENTAL CONDITIONS DURING THE HOLOCENE IN THE EASTERN ITALIAN ALPS USING A NOVEL XRF AND ICP-MS CALIBRATION METHOD  
*L. Poto<sup>1,2</sup>, J. Gabrieli<sup>2</sup>, G. Cozzi<sup>2</sup>, C. Turetta<sup>2</sup>, P. Ferretti<sup>2</sup>, S. Crowhurst<sup>3</sup>, C. Zaccone<sup>4</sup>, C. Barbante<sup>1,2</sup> (<sup>1</sup>University Ca' Foscari of Venice, <sup>2</sup>IDPA-CNR, Venice, <sup>3</sup>University of Cambridge, UK, <sup>4</sup>University of Foggia)*
- 16:10-16:30 ENHANCED HOLLOW FIBER FLOW FIELD-FLOW FRACTIONATION FOR THE ANALYSIS OF COMPLEX PROTEIN SAMPLES  
*A. Zattoni,<sup>1,2</sup> M. Tanase,<sup>1</sup> B. Roda,<sup>1,2</sup> P. Reschiglian,<sup>1,2</sup> L. Santambrogio<sup>3</sup> (<sup>1</sup>Dept. Chem. Univ. Bologna, byFlow, Bologna, <sup>2</sup>A. Einstein Coll. Chem., New York, USA)*
- 16:30-16:50 *Coffee Break*

PRESIEDE: L. SABBATINI (BARI)

### Oral Communications 37-42

- 16:50-17:10 RAPID DESORPTION ELECTROSPRAY IONIZATION-HIGH RESOLUTION MASS SPECTROMETRY-BASED METHOD FOR THE ANALYSIS OF MELAMINE MIGRATION FROM MELAMINE TABLEWARE  
*M. Mattarozzi, M. Milioli, C. Cavalieri, F. Bianchi, M. Careri (University of Parma)*
- 17:10-17:30 SURFACE CHEMISTRY OF Ni-FREE STAINLESS STEEL  
*M. Pisu, D. Addari, M. Fantauzzi, B. Elsener, A. Rossi (University of Cagliari)*

- 17:30-17:50 PE-CVD AS A POWERFUL TOOL FOR P3HT SURFACE MODIFICATION IN EGOFET BIOSENSORS DEVELOPMENT  
*M. Magliulo<sup>1</sup>, B.R. Pistillo<sup>1</sup>, M.Y. Mulla<sup>1</sup>, D. De Tullio<sup>1</sup>, K. Manoli<sup>1</sup>, N. Cioffi<sup>1</sup>, L. Sabbatini<sup>1</sup>, A. Mallardi<sup>2</sup>, G. Palazzo<sup>1</sup>, P. Favia<sup>1</sup>, L. Torsi<sup>1</sup> (1“A. Moro” University of Bari, 2CNR-IPCF, Bari)*
- 17:50-18:10 ELECTROCHEMICAL IMMUNOASSAY FOR CA125 DETECTION BASED ON SILVER-ENHANCED GOLD NANOPARTICLE LABEL  
*A. Ravalli<sup>1</sup>, Z. Taleat<sup>2</sup>, G.a Marrazza<sup>1</sup> (1University of Florence, 2Yazd University, Iran)*
- 18:10-18:30 PULSED ELECTRODEPOSITION OF NICKEL/PALLADIUM BINARY CODEPOSIT FROM GLUCONATE ALKALINE BATH. AN ELECTROCHEMICAL, XPS AND SEM INVESTIGATION
- 18:30-18:50 *I.G. Casella, M. Contursi (University of Basilicata)*  
A NEW OFET DEVICE CONFIGURATION FOR HIGHLY PERFORMING BIO-ELECTRONIC SENSORS  
*L. Torsi<sup>1</sup>, M. Magliulo<sup>1</sup>, A. Mallardi<sup>2</sup>, N. Cioffi<sup>1</sup>, L. Sabbatini<sup>1</sup>, G. Scamarcio<sup>1</sup>, G. Palazzo<sup>1</sup> (1University of Bari, 2CNR-IPCF, Bari)*

## Sessione Parallela 8. Miscellanea II

SALA B

PRESIEDE: M.P. COLOMBINI (PISA)

### Oral Communications 43-48

- 14:30-14:50 MULTIVARIATE STRATEGIES FOR SCREENING EVALUATION OF CHRONIC ALCOHOL ABUSE  
*V. Pirro<sup>1,2</sup>, P. Oliveri<sup>3</sup>, A. Salomone<sup>2</sup>, S. Lanteri<sup>3</sup>, M. Vincenti<sup>1,2</sup> (1Turin University, 2Antidoping Regional Center “A. Bertinaria”, Turin, 3University of Genoa)*
- 14:50-15:10 MULTIVARIATE CHEMICAL MAPS FROM  $\mu$ -FTIR AND DESI-MS HYPERSPECTRAL DATA  
*P. Oliveri<sup>1</sup>, G. Sciutto<sup>2</sup>, V. Pirro<sup>3</sup>, S. Prati<sup>2</sup>, R. Mazzeo<sup>2</sup>, L.S. Eberlin<sup>4</sup>, R.G. Cooks<sup>4</sup> (1University of Genoa, 2University of Bologna, 3 University of Turin, 4Purdue University, USA)*
- 15:10-15:30 ANALYTICAL CHARACTERIZATION OF THE OLEORESIN OF *COPAIFERA LANGSDORFFII* DESF. (FABACEAE)  
*F. Gelmini<sup>1</sup>, G. Beretta<sup>1</sup>, R. Mendichi<sup>2</sup>, A. Giacometti Schieron<sup>2</sup>, R. Maffei Facino<sup>1</sup> (1University of Milan, 2CNR, Milan)*
- 15.30-15:50 DIRECT ANALYSIS IN REAL TIME MASS SPECTROMETRY FOR THE NON-INVASIVE IDENTIFICATION OF CONSERVATION TREATMENTS OF THE DEAD SEA SCROLLS  
*M. Manfredi<sup>1</sup>, E. Marengo<sup>1</sup>, E. Robotti<sup>1</sup>, E. Mazzucco<sup>1</sup>, F. Gosetti<sup>1</sup>, G. Bearman<sup>2</sup>, F. France<sup>3</sup> and P. Shor<sup>4</sup> (1University of Piemonte Orientale, 2ANE Image, Pasadena, CA, USA, 3Library of Congress, Washington, DC, USA, 4Israel Antiq. Authority, Jerusalem)*
- 15:50-16:10 ICP-AES ANALYSIS OF BYZANTINE ANONYMOUS COPPER COINS FROM THE XI CENTURY AND COMPARISON WITH MICRO-EDXRF NON-DESTRUCTIVE ANALYSIS  
*G. Adami, M. Crosera, E. Baracchini, B. Callegher (University of Trieste)*

- 16:10-16:30 A HS-SPME-GCMS STUDY OF ROMANIAN AND BALTIC AMBER  
*I.D. van der Werf<sup>1</sup>, A. Aresta<sup>1</sup>, G.I. Truică<sup>2</sup>, G.L. Radu<sup>2</sup>, L. Sabbatini<sup>1</sup> (<sup>1</sup>University of Bari "Aldo Moro", <sup>2</sup>Politehnica University of Bucharest, Romania)*
- 16:30-16:50 *Coffee Break*
- PRESIEDE: C. BARBANTE (VENEZIA)
- Oral Communications 49-54**
- 16:50-17:10 CHARACTERIZATION OF TRACE ELEMENTAL COMPOSITION IN PM<sub>10</sub> SAMPLES MONITORED IN THE CITIES OF PIEDMONT REGION (ITALY)  
*A. Giacomino, M. Malandrino, O. Abollino, I. Zelano (University of Turin)*
- 17:10-17:30 MERCURY ISOTOPE RATIOS AS CONTAMINATION MARKERS: PROCEDURE DEVELOPMENT AND APPLICATIONS  
*C. Baschieri<sup>1</sup>, C. Durante<sup>1</sup>, A. Marchetti<sup>1</sup>, A. Berni<sup>1</sup>, L. Bertacchini<sup>1</sup>, S. Covelli<sup>2</sup>, R. Petrini<sup>2</sup>, A. Emili<sup>2</sup> (<sup>1</sup>University of Modena and Reggio Emilia, <sup>2</sup>University of Trieste)*
- 17:30-17:50 DETERMINATION OF WATER CONTENT IN ATMOSPHERIC PARTICULATE MATTER  
*S. Canepari<sup>1</sup>, C. Perrino<sup>2</sup>, C. Farao<sup>1</sup>, E. Marconi<sup>1</sup>, M.L. Astolfi<sup>1</sup> (<sup>1</sup>Sapienza University of Rome, <sup>2</sup>CNR, Rome)*
- 17:50-18:10 DETERMINATION OF Cd, Pb AND Cu IN SPRING WATERS OF THE SIBYLLINE MOUNTAINS NATIONAL PARK (CENTRAL ITALY) BY SQUARE WAVE ANODIC STRIPPING VOLTAMMETRY  
*C. Truzzi, A. Annibaldi, S. Illuminati, C. Finale, G. Scarponi (Politechnic University of Marche Region)*
- 18:10-18:30 STUDY OF HUMAN FOSSIL BONES FROM AN ARCHAEOLOGICAL SITE OF MIDDLE NILE BY TG, DTG AND ICP SPECTROSCOPY  
*M. Tomassetti, F. Marini, L. Campanella, A. Coppa (Sapienza University of Rome)*
- 18:30-18:50 STIR BAR SORPTIVE EXTRACTION AND LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY: A RAPID METHOD FOR TRACE ANALYSIS OF UV FILTERS IN DIFFERENT WATER MATRICES  
*E. Magi, M. Di Carro, K. T. N. Nguyen, C. Scapolla (University of Genoa)*
- 20:00 *Cena*
- 21:30 *Revealing Ravel: la scienza racconta il Bolero – Conferenza-spettacolo di L. Dei*

Giorno 4 • Mercoledì, 19 Settembre 2012

## Sessione Plenaria 4

SALA A

PRESIEDE: M. VINCENTI (TORINO)

09:00-09:40 **Conferenza Plenaria 4**

EVOLUTION OF THE ANALYTICAL STRATEGIES FOR THE  
DETECTION OF DOPING SUBSTANCES AND METHODS IN  
SPORT

*F. Botré<sup>1,2</sup>, X. de la Torre<sup>2</sup> (<sup>1</sup>University of Rome "La Sapienza",  
<sup>2</sup>Federazione Medico Sportiva Italiana)*

## Sessione Parallela 9. Ambiente e Beni Culturali

SALA A

PRESIEDE: R. FUOCO (PISA)

09:50-10:20 **Keynote Lecture 7**

NEW INSIGHTS ON THE INTERACTIONS BETWEEN  
ENVIRONMENT AND CULTURAL HERITAGE

*C. Barbante<sup>1,2</sup>, M.P. Colombini<sup>3</sup> (<sup>1</sup>University of Venice,  
<sup>2</sup>IDPA/CNR, Venice, <sup>3</sup>University of Pisa)*

**Oral Communications 55-59**

10:20-10:40 TOTAL INTRODUCTION OF MICROSAMPLES IN  
INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY  
BY HIGH-TEMPERATURE EVAPORATION CHAMBER WITH  
A SHEATHING GAS STREAM

*M. Grotti<sup>1</sup>, F. Ardini<sup>1</sup>, J.L. Todoli<sup>2</sup> (<sup>1</sup>University of Genoa,  
<sup>2</sup>University of Alicante, Spain)*

10:40-11:00 A STUDY ON ALKYD PAINT MEDIA BY GC/MS AND HPLC-  
ESI-Q/TOFMS

*J. La Nasa, I. Degano, F. Modugno, M.P. Colombini (University of  
Pisa)*

11:00-11:20 *Coffee Break*

11:20-11:40 AEROSOL CHARACTERIZATION BY PMF ANALYSIS OF  
SINGLE PARTICLE ATOFMS SPECTRA

*C. Giorio<sup>1</sup>, A. Tapparo<sup>1</sup>, M. Dall'Osto<sup>2</sup>, R.M. Harrison<sup>3</sup>, D.C.S.  
Beddows<sup>3</sup>, E. Nemitz<sup>4</sup>, C. Di Marco<sup>4</sup>, F. Canonaco<sup>5</sup>, A.S.H. Prévôt<sup>5</sup>  
(<sup>1</sup>University of Padua, <sup>2</sup>CSIC, Barcelona, Spain, <sup>3</sup>Birmingham  
University, UK, <sup>4</sup>Centre for Ecol. & Hydrol., Edinburgh, UK, <sup>5</sup>P.  
Scherrer Inst., Villigen, Switzerland)*

11:40-12:00 DEVELOPMENT OF A PORTABLE DEVICE FOR THE  
IDENTIFICATION OF OVALBUMIN IN PAINTING SAMPLES  
BY CHEMILUMINESCENT IMMUNOCHEMICAL CONTACT  
IMAGING

*M. Zangheri<sup>1</sup>, G. Sciutto<sup>1</sup>, M. Guardigli<sup>1</sup>, S. Prati<sup>2</sup>, M. Mirasoli<sup>1</sup>,  
R. Mazzeo<sup>2</sup>, A. Roda<sup>1</sup> (<sup>1</sup>University of Bologna, <sup>2</sup>University of  
Bologna, Ravenna Campus)*

12:00-12:20 STRONG COMPLEXATION OF LEAD(II) BY FULVIC SUBSTANCES UNDER ENVIRONMENTAL RELEVANT CONDITIONS  
*M. Pesavento, G. Alberti, R. Biesuz, G. D'Agostino (University of Pavia)*

## Sessione Parallela 10. Tossicologia Analitica e Chimica Analitica Forense

SALA B

PRESIEDE: F. BOTRÉ (ROMA)

09:50-10:20 **Keynote Lecture 8**

KEY ISSUES IN MODERN ANALYTICAL TOXICOLOGY

*M. Vincenti, University of Turin & Antidoping Regional Center, Turin*

WHERE IS AND WHERE IS GOING FORENSIC ANALYTICAL CHEMISTRY

*G. Mori, University of Parma*

**Oral Communications 60-64**

10:20-10:40 INNOVATIVE UHPLC-MS/MS STRATEGIES FOR THE DETECTION OF DRUGS OF ABUSE, PHARMACEUTICAL DRUGS AND METABOLITES IN FORENSIC INVESTIGATIONS

*A. Salomone<sup>1</sup>, D. Di Corcia<sup>1</sup>, E. Gerace<sup>1</sup>, V. Pirro<sup>1,2</sup> and M. Vincenti<sup>1,2</sup>(<sup>1</sup>Antidoping Regional Center "A. Bertinaria", Turin, <sup>2</sup>University of Turin)*

10:40-11:00 WHOLE-CELL BIOLUMINESCENT BIOSENSORS: A NEW WEAPON IN THE FIGHT AGAINST DOPING

*E. Michellini<sup>1,2</sup>, L. Cevenini<sup>1,2</sup>, L. Ekström<sup>3</sup>, J. Schulze<sup>3</sup>, M. Garle<sup>3</sup>, A. Rane<sup>3</sup>, M. D'Elia<sup>4</sup>, A. Roda<sup>1,2</sup>(<sup>1</sup>University of Bologna, <sup>2</sup>INBB, Rome, <sup>3</sup>Karolinska Inst., Stockholm, Sweden, <sup>4</sup>Gabinetto Reg. Polizia Sci. per l'Emilia Romagna, Bologna)*

11:00-11:20 *Coffee Break*

11:20-11:40 NEUTRAL LOSS AND PRECURSOR ION SCAN FOR THE SCREENING OF METHYLENEDIOXYAMPHETAMINE- AND PIPERAZINE-DERIVED DESIGNER DRUGS IN URINE BY LC-MS/MS

*C. Montesano<sup>1</sup>, M. Sergi<sup>2</sup>, M. Moro<sup>1</sup>, D. Compagnone<sup>2</sup>, R. Curini<sup>1</sup>(<sup>1</sup>"La Sapienza" University of Rome, <sup>2</sup>University of Teramo)*

11:40-12:00 IDENTIFICATION OF NEW DRUGS AND CREATION OF COMPOUND DATABASE FOR TIME OF FLY BASED TARGET SCREENING IN FORENSIC APPLICATION

*A. Gregori, F. Damiano (Reparto Carabinieri Investigazioni Scientifiche di Parma)*

12:00-12:20 SCIENCE AND CONSCIENCE ON THE COURT: THE DATING OF HANDWRITTEN DOCUMENTS

*G. Mori, M. Giannetto, A. Masutti, V. Trolla (University of Parma)*

## Workshop 3

SALA A

PRESIEDE: P. RESCHIGLIAN (BOLOGNA)

12:20-13:00 PUSH THE LIMITS IN MASS SPECTROMETRY

*M. Biglietto, AB Sciex*

13:00-14:30 *Lunch Break*

14:30-16:00 **Sessione Poster II**

15:40-16:00 *Coffee*

## Sessione Parallela 11. Bioanalitica e Omics

SALA A

PRESIEDE: G. SPOTO

16:00-16:30 **Keynote Lecture 9**

BIOANALYTICA, BIOSENSORS, AND WELL BEING

*G. Palleschi, D. Moscone, F. Arduini, L. Micheli, S. Piermarini, F. Ricci, F. Valentini, G. Volpe, Università di Roma Tor Vergata*

**Oral Communications 65-70**

16:30-16:50 TANDEM MASS SPECTROMETRY OF SULPHUR-CONTAINING GLYCOLIPIDS: A STEP FORWARD TOWARDS THE REGIOCHEMICAL ASSIGNMENT OF FATTY ACID ACYL CHAINS

*R. Zianni<sup>1</sup>, G. Bianco<sup>2</sup>, F. Lelario<sup>2</sup>, I. Losito<sup>1</sup>, F. Palmisano<sup>1</sup>, T.R.I. Cataldi<sup>1</sup> (<sup>1</sup>University of Bari, <sup>2</sup>University of Basilicata)*

16:50-17:10 NOVEL DIOXETANE-DOPED SILICA NANOPARTICLES AS ULTRASENSITIVE REAGENTLESS THERMOCHEMILUMINESCENT LABELS FOR BIOSENSING

*A. Roda<sup>1</sup>, M. Di Fusco<sup>1,2</sup>, A. Quintavalla<sup>1</sup>, M. Guardigli<sup>1</sup>, M. Mirasoli<sup>1,2</sup>, M. Lombardo<sup>1</sup>, C. Trombini<sup>1</sup> (<sup>1</sup>Bologna Univ., Chemistry Dept., <sup>2</sup>Bologna Univ., Interdept. Center for Ind. Res.)*

17:10-17:30 IDENTIFICATION OF GENES DYSREGULATION IN DIAMOND-BLACKFAN ANEMIA THROUGH GENOMICS AND MULTIVARIATE DATA ANALYSIS

*A. Aspesi<sup>1</sup>, E. Pavesi<sup>1</sup>, E. Robotti<sup>2</sup>, P. Roncaglia<sup>3</sup>, R. Crescitelli<sup>1</sup>, F. Avondo<sup>1</sup>, I. Boria<sup>1</sup>, L. Da Costa<sup>4</sup>, H. Moniz<sup>4</sup>, N. Mohandas<sup>5</sup>, U. Ramenghi<sup>6</sup>, A. Ronchi<sup>7</sup>, A. Follenzi<sup>1</sup>, E. Marengo<sup>2</sup>, S.R. Ellis<sup>8</sup>, S. Gustincich<sup>3</sup>, C. Santoro<sup>1</sup>, I. Dianzani<sup>1</sup> (<sup>1</sup>IRCAD, Piemonte Orientale Univ., <sup>2</sup>University of Piemonte Orientale., <sup>3</sup>SISSA/ISAS, Trieste, <sup>4</sup>Hop. R. Debre, Paris, France, <sup>5</sup>New York Blood Cntr., <sup>6</sup>Turin Univ., <sup>7</sup>Bicocca Milan Univ., <sup>8</sup>Louisville Univ., KY, USA)*

17:30-17:50 PROTEOMIC ANALYSIS OF PLATELET MICROPARTICLES BY NANO-HPLC/HIGH RESOLUTION MASS SPECTROMETRY

*A. L. Capriotti, G. Caruso, C. Cavaliere, A. Laganà, S. Piovesana, R. Samperi ("La Sapienza" University of Rome)*

Sezione Gruppo Inter-divisionale Biotech

17:50-18:10 BIOANALYTICAL ASSAYS FOR DNA-B[a]PDE ADDUCTS DETECTION

*V. Lanzone<sup>1</sup>, R. Tofalo<sup>1</sup>, G. Perpetuini<sup>1</sup>, M. Minunni<sup>2</sup>, S. Scarano<sup>2</sup>, F. Corrado<sup>3</sup>, M. Esposito<sup>3</sup>, D. Compagnone<sup>1</sup> (<sup>1</sup>Teramo University, <sup>2</sup>Florence University)*



- 18:10-18:30 METABOLOMICS OF TRANSGENIC PLANTS UNDER CHEMICAL STRESS.  
*R. Fuoco<sup>1</sup>, P. Bogani<sup>2</sup>, G. Capodaglio<sup>3</sup>, M. Del Bubba<sup>4</sup>, E. Magi<sup>5</sup>, O. Abollino<sup>4</sup>, S. Giannarelli<sup>1</sup>, M. M. Spiriti<sup>2</sup>, B. Muscatello<sup>1</sup>, S. Doumett<sup>4</sup>, C. Turetta<sup>3</sup>, R. Zangrando<sup>3</sup>, V. Zelano<sup>4</sup>, M. Grotti<sup>5</sup>, M. Di Carro<sup>5</sup>, M. Buiatti<sup>2</sup> (<sup>1</sup>University of Pisa, <sup>2</sup>Dept. Evol. Biol., Florence Univ., <sup>3</sup>Venice University, <sup>4</sup>Dept. Chem., Florence Univ., <sup>5</sup>Genoa University)*

## Sessione Parallela 12. Nanoanalitica

SALA B

PRESIEDE: R. SEEBER (MODENA)

- 16:00-16:30 **Keynote Lecture 10**  
 NANOANALYTICS : ANALYSIS OF “NANO-MATERIALS” AND “NANO-MATERIALS” FOR ANALYSIS. STATE OF ART AND PERSPECTIVES

*L. Sabbatini, University of Bari*

### Oral Communications 71-76

- 16:30-16:50 COUPLING FLOW FIELD-FLOW FRACTIONATION WITH PHOTOLUMINESCENCE SPECTROSCOPY FOR THE CHARACTERIZATION OF MULTIFUNCTIONAL NANOMATERIALS  
*P. Reschiglian<sup>1,2</sup>, F. Borghi<sup>1</sup>, B. Roda<sup>1,2</sup>, A. Zattoni<sup>1,2</sup>, R. Anand<sup>3</sup>, S. Monti<sup>3</sup> (<sup>1</sup>University of Bologna, <sup>2</sup>byFlow, Bologna, <sup>3</sup>CNR, Bologna)*
- 16:50-17:10 STRATEGY TO TUNE EXTEND AND NARROW THE DYNAMIC RANGE OF APTAMER-BASED SENSOR  
*A. Porchetta<sup>1,2</sup>, A. Vallée-Bélisle<sup>3</sup>, K.W. Plaxco<sup>3</sup>, G. Palleschi<sup>1,2</sup>, F. Ricci<sup>1,2</sup> (<sup>1</sup>University of Rome, Tor Vergata, <sup>2</sup>INBB, Rome, <sup>3</sup>University of California, USA)*
- 17:10-17:30 SURFACE ANALYTICAL CHARACTERIZATION OF MULTIFUNCTIONAL ZnO<sub>x</sub>-FLUOROPOLYMER NANO-COATINGS FOR THE TEXTILE INDUSTRY  
*C. Sportelli, M.A. Nitti, M. Valentini, E. Bonerba, G. Casamassima, L. Sabbatini, G. Tantillo, A. Valentini, N. Cioffi (University of Bari)*
- 17:30-17:50 GROWTH OF SULPHIDE THIN FILMS WITH TECHNOLOGICAL INTEREST  
*S. Cinotti, I. Bencistà, M.L. Foresti, F. Di Benedetto, A. De Luca, M. Romanelli, M. Innocenti (University of Florence)*
- 17:50-18:10 NANOSTRUCTURED ENZYMATIC BIOSENSOR BASED ON FULLERENE AND GOLD NANOPARTICLES: PREPARATION, CHARACTERIZATION AND ANALYTICAL APPLICATIONS  
*M.L. Antonelli<sup>1</sup>, S. Cannistraro<sup>2</sup>, E. Coppari<sup>2</sup>, G. Favero<sup>1</sup>, C. Lanzellotto<sup>1</sup>, F. Mazzei<sup>1</sup>, C. Tortolini<sup>1</sup> (<sup>1</sup>Sapienza University of Rome, <sup>2</sup>University of Tuscia, Viterbo)*
- 18:10-18:30 ADVANCED DETECTION OF GENETIC DISORDERS BY SURFACE PLASMON RESONANCE IMAGING  
*R. D'Agata<sup>1</sup>, G. Breveglieri<sup>2</sup>, L. M. Zanolì<sup>3</sup>, M. Borgatti<sup>2</sup>, R. Gambari<sup>2</sup>, G. Spoto<sup>1,3</sup> (<sup>1</sup>University of Catania, <sup>2</sup>University of Ferrara, <sup>3</sup>CNR, Catania)*

SALE A, B, C, D, E, F

18:35 **Assemblee Gruppi Divisionali**

20:30 *Cena sociale con intrattenimento musicale (Hotel Hermitage, Ristorante Maitù)*

**Giorno 5 • Giovedì, 20 Settembre 2012**

## **Sessione Plenaria 5**

SALA A

PRESIEDE: G. PALLESCHI (ROMA)

09:00-09:40 **Conferenza Plenaria 5**

RAPID, CHEAP, LABEL-FREE DETECTION OF CANCER MARKERS USING NANOWIRES INTERFACED TO VIRUSES

*G.A. Weiss, R.M. Penner, University of California, USA*

## **Sessione Parallela 13. Spettroscopia Analitica**

SALA A

PRESIEDE: A. ROSSI (CAGLIARI)

09:50-10:20 **Keynote Lecture 11**

ANALYTICAL SPECTROSCOPY: STATE OF THE ART AND FUTURE PERSPECTIVES

*G. Spoto, University of Catania*

### **Oral Communications 77-81**

10:20-10:40 SPECTROSCOPIC CHARACTERIZATION OF A SOLID CATALYST FOR DIOXIRANE-MEDIATED HETEROGENEOUS EPOXIDATIONS

*D. Cafagna<sup>1</sup>, C. Annese<sup>1</sup>, L. D'Accolti<sup>1</sup>, C. Fusco<sup>2</sup>, E. De Giglio<sup>1</sup> (<sup>1</sup>University of Bari, <sup>2</sup>ICCOM-CNR, Bari)*

10:40-11:00 A NEW STRATEGY FOR PRESSED POWDER EYE SHADOWS ANALYSIS: ALLERGENIC METAL IONS CONTENT AND PARTICLE SIZE DISTRIBUTION OF THE INSOLUBLE MATTER

*C. Contado (University of Ferrara)*

11:00-11:20 *Coffee Break*

11:20-11:40 NON-DESTRUCTIVE DEPTH PROFILE RECONSTRUCTION OF BIO-ENGINEERED SURFACES BY PARALLEL ANGLE RESOLVED X-RAY PHOTOELECTRON SPECTROSCOPY

*R. Pilolli, N. Ditaranto, N. Cioffi, L. Sabbatini (University of Bari)*

11:40-12:00 ARSENIC REMOVAL BY INTERACTION WITH GYPSUM: EFFECT OF PH, AS (V) CONCENTRATION AND PARTICLE SIZE

*M. Fantauzzi, D. Atzei, F. Cocco, R. Mascia, A. Rossi (University of Cagliari)*

12:00-12:20 APTAMERS IN BIOSENSORS: RECENT ADVANCES AND POSSIBLE APPLICATIONS

*S. Scarano, F. Crispo, M.L. Ermini, S. Mariani, M. Minunni (University of Florence)*

## Sessione Parallela 14. Spettrometria di Massa

SALA B

PRESIEDE: M. CARERI (PARMA)

09:50-10:20 **Keynote Lecture 12**

DIRECT-EI LC-MS INTERFACE: CAN LIQUID CHROMATOGRAPHY AND ELECTRON IONIZATION MASS SPECTROMETRY WORK TOGETHER AGAIN?

*A. Cappiello, G. Famiglioni, P. Palma, V Termopoli ("Carlo Bo" University of Urbino)*

**Oral Communications 82-86**

10:20-10:40 CHCA-BASED NEW MATRICES FOR MALDI-MS ANALYSIS OF LIPIDS AND PEPTIDES

*C. D. Calvano, A. Monopoli (University of Bari)*

10:40-11:00 SYNTHESIS AND CHARACTERIZATION OF TAILORED SURFACES AS SUPPORTS FOR DESORPTION ELECTROSPRAY IONIZATION-MASS SPECTROMETRIC INVESTIGATIONS

*A. Penna<sup>1</sup>, A. Rossi<sup>2,3</sup>, M. Careri<sup>1</sup>, N. D. Spencer<sup>3</sup> (<sup>1</sup>University of Parma, <sup>2</sup>University of Cagliari, <sup>3</sup>ETH Zurich, Switzerland)*

11:00-11:20 *Coffee Break*

11:20-11:40 DEVELOPMENT OF AN IMPROVED PERFORMANCES PROTOTYPE CHAMBER FOR LASER ABLATION ICP-QMS

*D. Monticelli<sup>1</sup>, D. Civati<sup>1</sup>, C. Dossi<sup>2</sup>, S. Recchia<sup>1</sup> (<sup>1</sup>Insubria University, Como, <sup>2</sup>Insubria University, Varese)*

11:40-12:00 NON-TARGET SCREENING OF THE PHOTODEGRADATION PRODUCTS FORMED IN A BEVERAGE CONTAINING ALLURA RED DYE

*F. Gosetti<sup>1</sup>, U. Chiuminatto<sup>2</sup>, E. Mazzucco<sup>1</sup>, G. Calabrese<sup>1</sup>, M.C. Gennaro<sup>1</sup>, E. Marengo<sup>1</sup> (Univ. of Piemonte Orientale, AB Sciex)*

12:00-12:20 RAPID EXTRACTION METHOD FOR GC/MS DETECTION OF ENVIRONMENTAL POLLUTANT RESIDUES IN HUMAN FETAL AND NEWBORN TISSUES

*V. Termopoli<sup>1</sup>, P. Palma<sup>1</sup>, G. Famiglioni<sup>1</sup>, A.M. Lavezzi<sup>2</sup>, L. Matturri<sup>2</sup>, A. Cappiello<sup>1</sup> (<sup>1</sup>Urbino University, <sup>2</sup>Milan University)*

SALA A

12:20 *Chiusura del Convegno (G. Arena, Università di Catania)*

12:40 *Pranzo*





Società Chimica Italiana

XXIII Congresso Nazionale della Divisione di Chimica Analitica  
Isola d'Elba, 16-20 Settembre 2012

*La Chimica Analitica per la vita e per l'ambiente*

## **SESSIONE POSTER I - Lunedì 17 settembre 14:30-16:00**

**Alimenti – Ambiente – Chemiometria – Sensori ed Elettroanalisi –  
Equilibri in Soluzione e Speciazione - Miscellanea**

**P1** A. Natale, C. Palermo, D. Nardiello, D. Centonze: A NEW ANALYTICAL METHOD FOR THE DETERMINATION OF POLYPHENOLS BY LIQUID CHROMATOGRAPHY AND PULSED AMPEROMETRIC DETECTION AT GLASSY CARBON ELECTRODES

*Department of Agro - Environmental, Science, Chemistry and Plant Protection, University of Foggia*

**P2** N. Tuzzolino, P. Censi, A. Pisciotta, F. Saiano: 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? – PART II

*DiSTeM, University of Palermo*

**P3** R. Toniolo, A. Pizzariello, N. Dossi, O. Abollino, G. Bontempelli: ROOM TEMPERATURE IONIC LIQUIDS AS PROFITABLE OVERLAYERS FOR THE QUARTZ CRYSTAL MICROBALANCE ESTIMATION OF FOOD QUALITY BY THEIR ODOR ANALYSIS

*Department of Food Science, University of Udine*

**P4** M. Tomassetti, T. Gatta, E. Mazzone, L. Campanella, R. Gabbianelli: MEASUREMENTS OF ANTIOXIDANT CAPACITY OF FRESH BLUEBERRY AND BLUEBERRY BASED INTEGRATORS, USING BIOSENSOR, SPECTROPHOTOMETRIC AND FLUORIMETRIC METHODS

*Department of Chemistry, University of Rome "La Sapienza"*

**P5** M. Tomassetti, E. Martini, L. Campanella, G. Favero, G. Sanzò, F. Mazzei: LACTOFERRIN DETERMINATION USING IMMUNOSENSOR METHOD BASED ON SURFACE PLASMON RESONANCE (AND TWO DIFFERENT TYPES OF MEASUREMENTS). COMPARISON WITH PREVIOUS IMMUNOLOGICAL METHODS

*Department of Chemistry, University of Rome "La Sapienza"*

**P6** C. Truzzi, S. Illuminati, A. Annibaldi, C. Finale, G. Scarponi: DETERMINATION OF PROLINE IN HONEY: COMPARISON BETWEEN OFFICIAL METHODS AND OPTIMIZATION OF THE ANALYTICAL METHODOLOGY

*Department of Life and Environmental Sciences, Università Politecnica delle Marche*

**P7** A. Russo, C. Benincasa, E. Perri: ISOTOPIC RATIO MASS SPECTROMETRY (IRMS) FOR CHARACTERIZING ORGANIC OLIVES

*CRA-OLI – Centro di Ricerca per l'Olivicoltura e l'Industria Olearia, Rende, Cosenza*

**P8** L. Pigani, A. Ulrici, G. Foca, F. Terzi, C. Zanardi, B. Zangognini, R. Seeber: MODIFIED ELECTRODES AND MICROELECTRODES FOR THE ANALYSIS OF FOOD MATRICES. DEVELOPMENT OF AN ELECTRONIC TONGUE

*Department of Chemistry, University of Modena and Reggio Emilia*

**P9** M. Del Carlo, V. Lanzone, D. Compagnone, L. Pigani, R. Seeber: ELECTROCHEMICAL EVALUATION OF EXTRAVERGIN OLIVE OIL'S TOCOPHEROL AND POLYPHENOLS IN ORGANIC PHASE

*Department of Food Sciences, University of Teramo*

- P10** M. Del Carlo, D. Pizzoni, G. C. Fusella, M. Alessandrini, D. Compagnone: EVALUATION OF THE ANALYTICAL PERFORMANCES OF A PEPTIDE BASED ELECTRONIC NOSE TOWARDS SOME TYPICAL AROMA MOLECULES AND FOOD SAMPLES  
*Department of Food Sciences, University of Teramo*
- P11** D. Corradini, A. Bellincontro, A. De Rossi, F. Mencarelli, I. Nicoletti: INFLUENCE OF CULTURAL PRACTICE AND POSTHARVEST DRYING PROCESS ON OCCURRENCE AND CONTENT OF PHENOLIC COMPOUNDS IN GRAPE BERRIES  
*CNR, Institute of Chemical Methodologies, Montelibretti, Roma*
- P12** M. A. Euterpio, I. Pagano, A. L. Piccinelli, L. Rastrelli and C. Crescenzi: DETERMINATION OF PHENOLIC COMPOUNDS IN COMPLEX MATRICES  
*Departement of Pharmaceutical and Biomedical Science, University of Salerno*
- P13** A. Di Taranto, M. Iammarino: DEVELOPMENT AND VALIDATION OF HPLC METHODS FOR THE DETERMINATION OF FOOD ANTIOXIDANTS NOT ADMITTED IN FRESH MEAT PREPARATIONS  
*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata*
- P14** R. Flamini, F. De Marchi, M. De Rosso, A. Dalla Vedova, A. Panighel, M. Gardiman, L. Bavaresco: STUDY OF GRAPE METABOLOMICS BY "SUSPECTS SCREENING" ANALYSIS  
*CRA -VIT, Conegliano, Treviso*
- P15** D. dell'Oro, M. Iammarino, N. Bortone, A. E. Chiaravalle: RADIOSTRONTIUM ANALYSIS IN CHEESE SAMPLES: DEVELOPMENT AND VALIDATION OF A RADIOCHEMICAL METHOD BY LIQUID SCINTILLATION COUNTING (LSC)  
*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata*
- P16** G.Vinci, F. Botrè, R. Preti, C. Colamonici, A. Tieri: TRACING THE ORIGIN OF MILK AND MILK PRODUCTS: A SIMPLIFIED PROCEDURE FOR EXTRACTION/ISOLATION OF GLYCEROL FROM WHOLE MILK AND GC-IRMS ANALYSIS  
*Department of Management, University of Rome "La Sapienza"*
- P17** C. Bignardi, M. Mattarozzi, A. Penna, L. Elviri, M. Careri: TARGETED PROTEOMIC APPROACH FOR TRACE MULTI ALLERGEN DETECTION IN FOODS  
*Department of Chemistry, University of Parma*
- P18** C. Corradini, A. Cavazza, C. Bignardi, C. Lantano: EVALUATION OF SAFETY AND QUALITY OF FOOD BY CZE-MS  
*Department of Chemistry, University of Parma*
- P19** M. Contursi, D. Gioia, I.G. Casella: DEVELOPMENT OF AN ANALYTICAL METHOD LC/PAD FOR THE ANALYSIS OF SULFONAMIDES IN HOMOGENATES OF MEAT FOR BABIES  
*Department of Chemistry, University of Basilicata*
- P20** A.L. Capriotti, C. Cavaliere, P. Foglia, A. Laganà, R. Nescatelli, R. Samperi: A NEW MICRO LIQUID/LIQUID EXTRACTION, ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-QUADRUPOLE/TIME OF FLIGHT MASS SPECTROMETRY METHOD FOR THE CHARACTERIZATION OF PHENOLIC FRACTION OF OLIVE OIL  
*Department of Chemistry, University of Rome "La Sapienza"*
- P21** A.L. Capriotti, C. Cavaliere, P. Foglia, A. Laganà, S. Piovesana, R. Samperi: DEVELOPMENT OF A MULTICLASS METHODOLOGY FOR EXTRACTION AND LC-MS/MS DETERMINATION OF VETERINARY DRUGS AND MYCOTOXINS IN EGG  
*Department of Chemistry, University of Rome "La Sapienza"*
- P22** G. Bianco, R. Pascale, F. Lelario, S. A. Bufo, T.R.I. Cataldi: GLUCOSINOLATE PROFILE OF *BARBAREA VULGARIS* SEEDS EVALUATED BY LC-ESI-FTICR MASS SPECTROMETRY  
*Department of Chemistry, University of Bari "Aldo Moro"*

**P23** L. Sabatino, M. Scordino, R. Caruso, E. Chiappara, P. Traulo, G. Gagliano: LC/MS/MS DETECTION OF SHORT-CHAIN AMINES AND MORPHOLINE IN WAX FORMULATIONS FOR FRUIT COATING

*Ministero delle Politiche Agricole Alimentari e Forestali, Laboratorio di Catania*

**P24** C. Bianchini, M. Pasquali, D. Zane, A. Curulli: DETERMINATION OF CAFFEIC ACID IN WINE AT POLY(3,4-ETHYLENEDIOXY)THIOPHENE PLATINUM MODIFIED ELECTRODE: A PRELIMINARY STUDY

*CNR Istituto per lo Studio dei Materiali Nanostrutturati (ISMN), Roma*

**P25** E. Magi, M. Di Carro, L. Bono: COMPARISON OF DIFFERENT COCOA LIQUORS BY GC-MS AND LC-MS/MS

*Department of Chemistry and Industrial Chemistry, University of Genoa*

**P26** G. Beretta and R. Maffei Facino: IDENTIFICATION OF A COUNTERFEIT NUTRITIONAL SUPPLEMENTS ADULTERATED WITH THE ANTI-INFLAMMATORY DRUG NIMESULIDE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY - DIODE ARRAY DETECTION (HPLC-DAD) AND ATTENUATED TOTAL REFLECTANCE (ATR-FT-IR)

*Department of Pharmaceutical Sciences, University of Milan*

**P27** C. Baggiani, L. Anfossi, F. Biagioli, C. Giovannoli, C. Passini: SOLID PHASE EXTRACTION OF PENICILLINS FROM MILK THROUGH THE USE OF SACRIFICIAL SILICA BEADS AS SUPPORT FOR MOLECULAR IMPRINTING

*Department of Chemistry, University of Turin*

**P28** C. Baggiani, L. Anfossi, F. Biagioli, C. Giovannoli, C. Passini: SCREENING OF A COMBINATORIAL LIBRARY OF ORGANIC POLYMERS FOR THE SOLID PHASE EXTRACTION OF PATULIN FROM APPLE JUICE

*Department of Chemistry, University of Turin*

**P29** D. Naviglio: THE EXTRACTOR NAVIGLIO® IN FOOD PRODUCTIONS

*Department of Food Science, University of Naples Federico II*

**P30** S. Materazzi, F. Marini, M. Bevilacqua, R. Bucci: FOOD QUALITY CONTROL: APPLICATION OF NEAR INFRARED SPECTROSCOPY FOR DRIED EGG-PASTA CHARACTERIZATION

*Department of Chemistry, University of Rome "La Sapienza"*

**P31** M. Ciulu, R. Farre, I. Floris, A. Panzanelli, M.I. Pilo, N. Spano, G. Sanna: A RAPID HPLC METHOD FOR THE DETERMINATION OF FREE HMF IN ROYAL JELLY

*Dipartimento di Chimica e Farmacia, Università degli Studi di Sassari*

**P32** A. Spanu, L. Daga, A.M. Orlandoni, G. Sanna: THE ROLE OF IRRIGATION TECHNIQUES IN ARSENIC BIOACCUMULATION IN RICE (ORYZA SATIVA L.)

**P33** E. Venturini, I. Vassura, L. Ferroni, F. Passarini, E. Bernardi: SOURCE APPORTIONMENT STUDY NEAR A MSW INCINERATOR BY POSITIVE MATRIX FACTORIZATION (PMF)

*Department of Industrial Chemistry, University of Bologna*

**P34** S. Bogialli, F. Nigro Di Gregorio, L. Lucentini, A. Pivato, E. Ferretti, G. Favaro, P. Pastore, A. Tapparo: A CASE OF MANAGEMENT OF A TOXIC CYANOBACTERIUM BLOOM (*PLANKTOTHRIX RUBESCENS*) AFFECTING AN ITALIAN DRINKING WATER BASIN

*Department of Chemistry, University of Padua*

**P35** G. Carugati, A. Pozzi, C. Colombo, S. Terrana, R. Gambillara: CHARACTERIZATION OF THE CHEMICO-PHYSICAL AND HYDROGEOLOGICAL PARAMETERS OF SPRING WATERS IN THE AREA OF LIVIGNO (ITALY)

*Department of Science and High Technology, University of Insubria*

**P36** S. Berto, M. Isaia, E. De Laurentiis, D. Vione: SPECTRAL MODIFICATIONS OF LAKE AND SUBTERRANEAN WATERS UNDER IRRADIATION

*Department of Chemistry, University of Turin*

- P37** V. Lanzone, M. Mascini, M. Sergi, M. Del Carlo, F. Corrado, M. Esposito, D. Compagnone: SELECTION OF BIOMIMETIC RECEPTORS FOR DIOXINS: FROM THE RATIONAL DESIGN TO THE APPLICATION IN SOLID-PHASE EXTRACTION  
*Department of Food Sciences, University of Teramo*
- P38** P. Oliveri, M.C. Casolino, M. Casale, S. Lanteri, L. Medini, F. Mare: AUTHENTICITY ASSESSMENT OF COMMERCIAL OLIVES IN BRINE  
*Department of Pharmacy, University of Genoa*
- P39** A. Ulrici, C. Ferrari, G. Foca, D. Salvo, R. Seeber: EFFICIENT STRATEGIES FOR DATA COMPRESSION OF HYPERSPECTRAL IMAGES  
*Dipartimento di Scienze Agrarie e degli Alimenti, Università di Modena e Reggio Emilia*
- P40** A. Ulrici, M. Calderisi, F. Terzi, R. Seeber: CHEMOMETRIC METHODS FOR THE DEVELOPMENT OF A NEW SENSING SYSTEM FOR THE IDENTIFICATION OF DRUG PRECURSORS IN GASEOUS PHASE  
*Dipartimento di Scienze Agrarie e degli Alimenti, Università di Modena e Reggio Emilia*
- P41** V. Consonni, R. Todeschini, and M. Buscema: NEW NONLINEAR PERSPECTIVES IN CHEMOMETRICS: THE ATDM METHOD  
*Department of Environmental Sciences, University of Milano-Bicocca*
- P42** E. Robotti, M. Manfredi, L. Boatti, F. Marsano, A. Viarengo, E. Marengo: EVALUATION OF THE PERFORMANCE OF MALDI-TOF FOR QUANTITATIVE AND SEMIQUANTITATIVE DETERMINATIONS THROUGH MULTIVARIATE TOOLS  
*Department of Sciences and Technological Innovation, University of Piemonte Orientale*
- P43** S. Furlanetto, B. Pasquini, G. la Marca, S. Orlandini, Y. Vander Heyden, S. Pinzauti: CLASSIFICATION MODELS IN NEWBORN SCREENING FOR METABOLIC DISEASES BY LC-MS/MS  
*Department of Pharmaceutical Sciences, University of Florence*
- P44** B. Pasquini, G. Beretta, S. Orlandini, S. Pinzauti, S. Furlanetto: GEOGRAPHICAL CHARACTERISATION OF HONEYS BY A CHEMOMETRIC APPROACH: A COMPARATIVE STUDY AMONG CLASSIFICATION MODELS  
*Department of Pharmaceutical Sciences, University of Florence*
- P45** S. Orlandini, M. Bragagni, N. Mennini, P. Mura, S. Pinzauti, S. Furlanetto: MIXTURE DESIGN AND CAPILLARY ELECTROPHORESIS AS ANALYTICAL TOOLS FOR THE OPTIMIZATION OF FUNCTIONALIZED NIOSOMES  
*Department of Pharmaceutical Sciences, University of Florence*
- P46** N. Mennini, S. Orlandini, B. Pasquini, P. Mura, S. Pinzauti, S. Furlanetto: EXPERIMENTAL DESIGN AND CAPILLARY ELECTROPHORESIS IN THE DEVELOPMENT OF CYCLODEXTRIN-BASED HYDROGELS FOR ORAL ADMINISTRATION OF OXAPROZIN  
*Department of Pharmaceutical Sciences, University of Florence*
- P47** M. Crosera, P. Fornasiero, F.F. Larese, C. Bianco, G. Maina, G. Adami: DERMAL ABSORPTION OF PLATINUM GROUP ELEMENTS NANOPARTICLES FROM VEHICLES' CATALYSTS  
*Department of Chemical and Pharmaceutical Sciences, University of Trieste*
- P48** A. Giuffrida, F. Attanasio, I. Naletova, V. Muronetz, A. Sinopoli, G. Pappalardo, E. Rizzarelli: STUDY OF THE INTERACTION BETWEEN TREHALOSE CONJUGATED  $\beta$ -SHEET BREAKER PEPTIDE AND  $A\beta(1-42)$  MONOMERS.  
*CNR-Institute of Biostructure and Bioimaging, Catania*
- P49** E. Bramanti, S. Monti, A. Sgarbossa, R. Bizzarri, F. Lenci, V. Barone: THE DESTABILIZING ACTIVITY OF FERULIC ACID ON A-BETA PROTOFIBRILS. AN EXPERIMENTAL AND MOLECULAR DYNAMICS SIMULATIONS STUDY.  
*CNR-Istituto di Chimica dei Composti Organometallici, U.O. Pisa*

- P50** V. della Porta, S. Monti, M. Onor, A. Cepparrone, E. Bramanti: STUDY OF THE INTERACTION OF CHLORINATED AND SULFOCHLORINATED PARAFFINS WITH GELATIN B AND SKIN POWDER. A MODEL FOR FATTENING IN THE LEATHER TANNING PROCESS  
*CNR-Istituto di Chimica dei Composti Organometallici, U.O. Pisa*
- P51** S. Biagi, S. Ghimenti, V. della Porta, M. Onor, E. Bramanti: SIMULTANEOUS DETERMINATION OF LACTATE AND PYRUVATE IN HUMAN SWEAT: A NON INVASIVE APPROACH.  
*C.N.R., Istituto per i Processi Chimico-Fisici-IPCF-UOS Pisa*
- P52** A.L. Bianchera, R. Cucinelli, M. Asadzadeh, R. Bettini, L. Elviri: COMBINED CHITOSAN-SACCHARIDE SCAFFOLDS FOR CONTROLLED PEPTIDE RELEASE  
*Department of Pharmacy, Biopharmanet-TEC, University of Parma*
- P53** L. Elviri, A.L. Bianchera, M. Vigliano, M. Altieri, R. Bettini: LC-ESI-MS DETERMINATION OF HYALURONIC ACID IN DRUG RELEASE EXPERIMENTS FROM PIG INTESTINAL MUCOSA: AN EVALUATION OF MATRIX EFFECT  
*Department of Pharmacy, Biopharmanet-TEC, University of Parma*
- P54** A. Manfredi, M. V. Bianchi, L. Elviri, H. Rosenqvist, A. Rogowska-Wrzesinska, O. Nørregaard Jensen, E. Pilotti, C. Casoli: ANALYTICAL STRATEGIES FOR PHOSPHOPROTEIN ENRICHMENT FROM CELLS. A CASE STUDY: QUALITATIVE PHOSPHOPROTEOME OF CD4+ T-LYMPHOCYTES FROM NAÏVE HIV-1 INFECTED PATIENTS  
*Department of Pharmacy, Biopharmanet-TEC, University of Parma*
- P55** N. Ditaranto, A. Trapani, C. Palazzo, N. Cioffi, G. Trapani, L. Sabbatini: XPS CHARACTERIZATION OF CHITOSAN-BASED NANOPARTICLES FOR DRUG-DELIVERY APPLICATIONS  
*Department of Chemistry, University of Bari "Aldo Moro"*
- P56** F. Valentini, E. Ciambella, M. Carbone, G. Palleschi, M. Prato, M. Bonchio, N. Ditaranto and L. Sabbatini: SINGLE-WALL CARBON NANOHORNS FOR THE ASSEMBLY OF SCREEN-PRINTED ELECTRODES (SPEs)  
*Department of Chemistry, University of Bari "Aldo Moro"*
- P57** F. Valentini, D. Roscioli, M. Carbone, V. Conte, B. Floris, G. Palleschi, N. Ditaranto, L. Sabbatini: SYNTHESIS AND CHARACTERIZATION OF FUNCTIONALIZED GRAPHENE SHEETS WITH IONIC LIQUIDS (ILS)  
*Department of Chemistry, University of Bari "Aldo Moro"*
- P58** M.R. Guascito, D. Chirizzi, C. Malitesta, T. Siciliano, A. Tepore: BIMETALLIC Pt/Te MICROTUBES MODIFIED ELECTRODE FOR GLUCOSE AMPEROMETRIC DETECTION  
*Department of Di.S.Te.B.A., University of Salento*
- P59** A. Roda, L. Cevenini, E. Michellini, P. Simoni, M. Zangheri, B.R. Branchini: NEW METABOLICALLY BIOTINYLATED THERMOSTABLE RED- AND GREEN- EMITTING LUCIFERASES TO IMPROVE MULTIPLEXED BIO-CHEMILUMINESCENT BIOSENSORS  
*Department of Chemistry "G. Ciamician", University of Bologna*
- P60** D. Badocco, A. Mondin, P. Pastore: LIGHT INTENSITY BASED OXYGEN OPTICAL SENSOR USING A "SMART" DRIFT CORRECTION ALGORITHM  
*Dipartimento di Scienze Chimiche, Università di Padova*
- P61** A. Florea, A. Ravalli, C. Cristea, R. Sandulescu, G. Marrazza: ELECTROCHEMICAL IMMUNOASSAY FOR MUC1 DETECTION AS DIAGNOSTIC TOOLS IN OVARIAN CANCER  
*Department of Chemistry "Ugo Schiff", University of Florence*
- P62** A. Ravalli, L. Rivas, A. de la Escosura-Muñiz, J. Pons, A. Merkoçi, G. Marrazza: NEW APTASENSOR FOR VEGF BIOMARKER DETECTION  
*Department of Chemistry "Ugo Schiff", University of Florence*



- P63** D. Voccia, F. Bettazzi, G. Marrazza, I. Palchetti: ELECTROCHEMICAL BIOSENSING PLATFORMS FOR MICRORNA DETECTION  
*Department of Chemistry "Ugo Schiff", University of Florence*
- P64** E. Mazzotta, C. Malitesta, M. Díaz-Álvarez, A. Martín-Esteban: ENTRAPMENT OF MOLECULARLY IMPRINTED POLYMER MICROPARTICLES IN ELECTROPOLYMERIZED FILMS FOR ELECTROCHEMICAL SENSING APPLICATIONS  
*Department of Di.S.Te.B.A., University of Salento*
- P65** D. Merli, B. Sichel, D. Dondi, A. Profumo: CHARACTERIZATION OF A NEW POLYMERIC PHASE CONTAINING MWCNTs FOR ELECTROANALYTICAL APPLICATIONS  
*Department of Chemistry, University of Pavia*
- P66** G. D'Agostino, M. Pesavento, G. Alberti, R. Biesuz: SELECTIVE AMPEROMETRIC SENSOR FOR TNT BASED ON MOLECULAR IMPRINTED POLYMER AS ELECTROLYTIC MEDIUM  
*Department of Chemistry, University of Pavia*
- P67** Amendola, G. Alberti, G. Bergamaschi, R. Colleoni, G. d'Agostino, R. Biesuz: RECEPTORS IN SOLID PHASE: DEVELOPING SENSORS FOR ANIONIC RADIONUCLIDES  
*Department of Chemistry, University of Pavia*
- P68** G. Alberti, L. Magrino, R. Doi, M. Pesavento, R. Biesuz: SORPTION OF La(III) ON DIFFERENT SOLID PHASES  
*Department of Chemistry, University of Pavia*
- P69** F. Bettazzi, L. Enayati, I. Campos Sánchez, R. Motaghed, I. Palchetti: DEVELOPMENT OF AN ELECTROCHEMICAL AFFINITY BIOSENSOR FOR THE DETECTION OF TUMOR NECROSIS FACTOR ALPHA  
*Department of Chemistry "Ugo Schiff", University of Florence*
- P70** A. Benedetti, F. Canganella, R. Fani, S. Mocali, I. Palchetti: VOLTAMMETRIC CHARACTERIZATION OF SOIL-ISOLATED MICROBIAL COMMUNITY FOR THE DEVELOPMENT OF A MICROBIAL FUEL CELL  
*Department of Chemistry "Ugo Schiff", University of Florence*
- P71** D. Monticelli, S. Caprara, L. M. Laglera Baquer: AUTOMATION OF ULTRATRACE ANALYSIS OF IRON BY ADSORPTIVE STRIPPING VOLTAMMETRY  
*Department of Science and High Technology, University of Insubria*
- P72** D. Migliorelli, L. Cozzi, G. Volpe, C. Fulci, G. Palleschi, L. Croci: AN ELECTROCHEMICAL METHOD BASED ON SCREEN-PRINTED ELECTRODE COUPLED WITH AN HAEMOLYTIC ASSAY FOR Palytoxin DETECTION IN SEAFOOD  
*Department of Science and Chemical Technologies, Università "Tor Vergata"*
- P73** F. Maraschi, M. Sturini, A. Speltini, E. Rivagli, R. Calisto, A. Profumo, V. Caratto, M. Ferretti: PRECONCENTRATION AND DETERMINATION OF VANADIUM AS V(V), ON IMMOBILIZED NANOMETER TITANIUM DIOXIDE MICROCOLUMN  
*Department of Chemistry, University of Pavia*
- P74** L. Anfossi, C. Passini, C. Giovannoli, F. Biagioli, C. Baggiani: TOWARDS THE ESTABLISHMENT OF AN "ENZYME MOLECULAR IMPRINTED POLYMER -SORBENT ASSAY (E-MIP-SA)" FOR MEASURING THE PEPTIDE HORMONE HEPcidin.  
*Department of Chemistry, University of Turin*
- P75** F. Biagioli, C. Baggiani, C. Giovannoli, L. Anfossi, C. Passini: A LATERAL FLOW IMMUNOASSAY FOR THE DETECTION OF Ochratoxin A IN WINES  
*Department of Chemistry, University of Turin*
- P76** C. Bretti, F. Crea, R.M. Cigala and G. Vianelli: THE EFFECT OF THE IONIC STRENGTH AND TEMPERATURE ON THE SOLUBILITY AND ACID-BASE PROPERTIES OF DOPAMINE IN NaCl<sub>(aq)</sub>  
*Department of Inorganic Chemistry, University of Messina*

**P77** F. Crea, C. De Stefano, A. Irto, G. Lando, D. Milea, S. Sammartano  
SPECIATION OF LANTHANOIDS(III) IN AQUEOUS SOLUTION: SEQUESTRATION BY  
REDUCED GLUTATHIONE

*Department of Inorganic Chemistry, University of Messina*

**P78** C. Foti, O. Giuffrè, S. Sammartano: ACID-BASE PROPERTIES AND COMPLEXING ABILITY  
OF ETIDRONIC ACID IN AQUEOUS SOLUTION

*Department of Inorganic Chemistry, University of Messina*

## **Sessione Poster II - Mercoledì 19 settembre 14:30-16:00**

**Bioanalitica – Tossicologia Analitica e Chimica Analitica Forense –  
Nanoanalitica – Ambiente e Beni Culturali – Spettrometria di Massa –  
Spettroscopia Analitica**

**P79** A.L. Capriotti, C. Cavaliere, P. Foglia, F. Gasparrini, A. Laganà, G. Pierri, P. Simone, O.  
Ursini, C. Villani: SEPARATION AND CHARACTERIZATION OF INTACT PROTEINS USING  
MONOLITHIC CAPILLARY COLUMNS WITH MS DETECTION: EFFECTS OF EXPERIMENTAL  
PARAMETERS ON SELECTIVITY, EFFICIENCY, MS SPECTRA QUALITY AND OVERALL  
THROUGHPUT

*Department of Chemistry, University of Rome "La Sapienza"*

**P80** A.L. Capriotti, G. Caruso, A. Laganà, S. Piovesana, R. Samperi: EVALUATION OF THREE  
DIFFERENT LOW MOLECULAR WEIGHT PROTEIN PREFRACTIONATION AND ENRICHMENT  
METHODS FOR PLASMA BIOMARKER DISCOVERY

*Department of Chemistry, University of Rome "La Sapienza"*

**P81** A. Naccarato, G. Sindona, A. Tagarelli: PAPER SPRAY IONIZATION TANDEM MASS  
SPECTROMETRY (PS-MS/MS) IN URINARY ACYLCARNITINES ASSAY

*Department of Chemistry, University of Calabria*

**P82** E. Gionfriddo, A. Naccarato, G. Sindona, A. Tagarelli: URINARY ACYLCARNITINES  
DETERMINATION BY GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY COUPLED  
WITH SOLID-PHASE MICROEXTRACTION (SPME-GC-MS/MS)

*Department of Chemistry, University of Calabria*

**P83** L.M. Zanolì, G. Alfonsino, R. D'Agata, G. Spoto: AN ISOTHERMAL AMPLIFICATION  
METHOD FOR DNA DETECTION IN DROPLET-BASED MICROFLUIDIC DEVICES

*Institute of Biostructure and Bioimaging, Catania*

**P84** G. Emma, Y.M. Ma, R. Hider: SELECTIVE QUANTIFICATION OF NTBI USING IRON  
SENSITIVE FLUORESCENT BEADS

*Department of Chemistry, University of Pavia*

**P85** F. Bonvicini, M. Mirasoli, E. Manaresi, G.A. Gentilomi, A. Roda, G. Gallinella:  
DEVELOPMENT OF BRDU-BASED CHEMILUMINESCENT ASSAYS FOR THE QUANTITATIVE  
DETECTION AND IMAGING OF CELLULAR AND B19 VIRAL LABELLED DNA IN INFECTED  
CELLS

*Department of Chemistry "G. Ciamician", University of Bologna*

**P86** M. Mirasoli, F. Bonvicini, E. Manaresi, F. Di Furio, M. Zangheri, G. Gallinella, A. Roda:  
ANALYSIS AT SINGLE CELL LEVEL OF PARVOVIRUS B19 EXPRESSION PROCESS BY  
CHEMILUMINESCENT MICROSCOPE IMAGING ASSAYS

*Department of Chemistry "G. Ciamician", University of Bologna*

- P87** M. Mirasoli, M. Zangheri, D. Caputo, G. de Cesare, R. Scipinotti, A. Nascetti, A. Roda: INTEGRATED HYDROGENATED AMORPHOUS SILICON PHOTOSENSORS FOR CHEMILUMINESCENCE DETECTION IN MICROFLUIDIC BIOANALYTICAL DEVICES  
*Department of Chemistry "G. Ciamician", University of Bologna*
- P88** C.S. Aman, G. Cighetti: VALIDATION OF AN HPLC-FLUORESCENCE METHOD FOR THE SIMULTANEOUS FREE AND GLYCOSYLATED PYRIDINIUM CROSSLINKS DETERMINATION IN URINE  
*Dipartimento di Scienze Biomediche e Cliniche "L. Sacco", Università degli Studi di Milano*
- P89** T. Lomonaco, S. Ghimenti, M. Onor, F. Di Francesco, A. Paolicchi, L. Ruocco, G. Pellegrini, M. G. Trivella, R. Fuoco: DETERMINATION OF BOUND AND UNBOUND WARFARIN AND ITS METABOLITES IN HUMAN PLASMA  
*Department of Chemistry, University of Pisa*
- P90** M. Monteleone, G. Sindona, A. Tagarelli: QUANTIFICATION OF HOMO VANILLIC ACID, VANILLYLMANDELIC ACID AND 5-HYDROXYINDOLEACETIC ACID IN HUMAN URINE BY SPME-GC-QqQ-MS AS MARKERS OF NEUROBLASTIC AND CARCINOID TUMORS  
*Department of Chemistry, University of Calabria*
- P91** G. Carlucci, A. Romagnoli, F. De Lutiis, M. Locatelli: HPLC-DAD METHOD FOR THE SIMULTANEOUS DETERMINATION OF PRULIFLOXACIN AND ULIFLOXACIN IN HUMAN PLASMA SAMPLES FOR STABILITY STUDIES  
*Dipartimento di Scienze del Farmaco, Università degli Studi "G. d'Annunzio" Chieti-Pescara*
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*Microchemistry and Microscopy Art Diagnostic Laboratory (M2ADL), University of Bologna*





## **Conferenze Plenarie**

## REACH REGULATION AND ANALYTICAL CHEMISTRY

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1. France

Over the next decades thousands of chemicals manufactured in or imported into the European Union will have to be tested, assessed and registered under the REACH regulation. Information on health and safety of these chemicals must be provided by production companies (manufacturers, users) to the European Chemicals Agency (ECHA) in Helsinki. In order to achieve a successful implementation of REACH, a comprehensive effort of fundamental research is requested.

The research needs in conjunction with the REACH Regulation will be presented and particularly the various aspects of the assessment of chemicals related to toxicology, ecotoxicology, QSAR and the role of advanced analytical chemistry. Analytical chemistry is present to some extent at every stage of the Chemicals Policy and will be strongly involved into the implementation of REACH. The major issues of concern will be the following:

- better analytical specificities of analysed compounds including degraded and biotransformed compounds in various environmental compartments,
- low limits of detection linked to effects at low dose exposure,
- multi-component analyses to reduce human and environmental impacts and costs.

### References

REACH regulation: <http://echa.europa.eu/> and  
<http://ec.europa.eu/enterprise/sectors/chemicals/reach/>

## RECENT DEVELOPMENTS IN ENANTIOSELECTIVE ANALYSIS

B. Chankvetadze

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In this presentation recent developments in the field of enantioselective chromatographic analysis are summarized. The emphasis is made on development of novel chiral stationary phases (CSP) for enantioseparations using high-performance liquid chromatography (HPLC) [1], nano-liquid chromatography (CLC) and capillary electrochromatography (CEC) [2,3]. In the first part of the presentation the emphasis will be made on novel phenylcarbamate derivatives of cellulose and amylose as useful CSPs for analytical and preparative scale enantioseparations. These novel materials are applicable for HPLC enantioseparations in combination with normal phase-, polar organic mobile phase and reversed-phase eluents, as well as for SFC enantioseparations at higher pressure. In the second part of the presentation the effect of fine tuning of the properties of these materials and separation conditions on resolution of enantiomers of various compounds will be discussed in detail using the examples of challenging separations. In the third part of the presentation some new developments and applications in nano-liquid chromatography and capillary electrochromatography will be discussed [2, 3]. In the final part of the presentation newly observed unusual effects in separation of enantiomers with polysaccharide-based chiral stationary phase such as reversal of the enantiomer elution order of some chiral drugs and amino acid derivatives by variation of separation temperature and composition of the mobile phase [4-6], HILIC-like behaviour of polysaccharide-based materials, enantioselective peak focusing phenomenon and unusual temperature-dependence of compound retention will be discussed.

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## COMPANION DIAGNOSTICS FOR PERSONALIZED MEDICINE

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Diagnostic tests used in selecting patients for treatment with a particular therapeutic have been termed companion diagnostics. These tests hold great promise for personalizing medicine and typically are diagnostic kits or Point-of-Care Testing (POCT) biosensor-like format. The analytical device should be highly flexible multiplex format and patients-tailored for the simultaneous assay of a panel of different biomarkers. A miniaturized self-standing device and the use of a detection principle characterized by a simple instrumentation and an analytical signal with a wide dynamic range of linearity allowing to perform in one run ultrasensitive immunoassays, nucleic acid hybridization reaction and simple enzymatic assays. Among the different detection principles, chemical luminescence-based techniques are particularly attractive, since they combine high detectability with the requirement of simple and compact instrumentation. Light emission from luminescent probes can be measured at the single photon level with CCD imaging devices or new generation photodiodes. To achieve ultrasensitive simultaneous detection of several analytes in one spot or tube a combination of different labels can be exploited, which light emission is sequentially generated by means of chemiluminescence-CL, bioluminescence -BL, electrogenerated luminescence-ECL and thermochemiluminescence-TCL triggering.

The device was constituted by a transparent three-electrode electrochemical cell based on indium/tin oxide (ITO)-coated glass, which allow triggering the ECL of  $\text{Ru}(\text{bpy})_3^{2+}$  labels in the presence of tripropylamine, resulting in light emission. The CL emission of a peroxidase-labeled probe and/or the BL emission of a luciferase-labeled probe could be also induced in the same cell by adding the appropriate BL-CL substrates. A second ITO coating layer at the bottom side of the cell was used to rapidly heat the glass up to  $100^\circ\text{C}$ , thus triggering the TCL emission of a dioxetane-labeled probe.

In addition to the separate triggering of the luminescence reactions, further multiplexing abilities can be achieved by exploiting spatial resolution imaging (when different analytes are captured in different positions and revealed by a common probe) and/or spectral resolution (when probes emitting at different wavelengths are used) and up to 4-8 probes can be measured in a miniaturized cartridge comprising a simple microfluidic system for reagents delivery.

The use of a sensitive cooled CCD in a lensless format, placed directly in contact with the cartridge via a fiber optic faceplate, allowed not only the quantification of the light emission from the different probes, but also the 2D imaging of the probes spotted in different areas of the glass cartridge.

As a feasibility study, the multiplexing capability has been demonstrated in different assay principles, including immuno- and nucleic acid assays, demonstrating adequate analytical performance.

## **EVOLUTION OF THE ANALYTICAL STRATEGIES FOR THE DETECTION OF DOPING SUBSTANCES AND METHODS IN SPORT**

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This communication presents a general overview on the activity of the antidoping laboratories accredited by the World Anti-Doping Agency (WADA), performing the analysis of the Athletes biological samples with the aim to detect the illicit recourse of performance-enhancing drugs and methods and of masking agents. The presentation outlines the evolution, over the last four decades, of the laboratory methods and techniques, progressively developed for the detection, in biological samples (urine and blood), of markers of the abuse of prohibited substances, with special emphasis on the most recent acquisition in the development of specific analytical procedures to tackle the new forms of sport doping. An overview on the present and future analytical challenges for the antidoping laboratories is presented.

The presentation also focuses on the specific issues of “masking agents”, that are substances or methods capable of “hiding” other forbidden substances, thus reducing the efficacy of the experimental strategies used to detect the abuse of doping agents by the analysis of biological fluids. Two representative cases will be discussed in details, that are the classes of “doping drug delivery systems”, capable of altering the window of detectability of forbidden substances, and that of “metabolic modulators”, i.e. specific inhibitors or inducers of the enzymatic systems responsible for the Phase-I and Phase-II metabolism of doping agents.

The pharmacologic relevance to the doping field and the analytical strategies developed to study these indirect forms of performance enhancing substances and methods will also be discussed.

## **RAPID, CHEAP, LABEL-FREE DETECTION OF CANCER MARKERS USING NANOWIRES INTERFACED TO VIRUSES**

G.A. Weiss, R.M. Penner,

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USA.

In this talk I'll describe a new approach to biosensors that has as its objective the development of ultra-cheap, disposable biosensors that are able to detect virtually any analyte molecule. The realization of this biosensor is made possible by two very new developments in our laboratory: The first is a nanowire fabrication technique called Lithographically Patterned Nanowire Electrodeposition (LPNE) that permits very long (> 1 cm), very uniform noble metal nanowires as small as 6 nm x 20 nm to be patterned on glass surfaces. Previously, such nanowires could only be obtained using electron beam lithography – a tedious and expensive fabrication method. The second is the demonstration that filamentous bacteriophage particles that have been engineered using phage display to selectively recognize and bind a particular analyte molecule can be covalently attached to metal surfaces. The resulting “covalent virus surfaces” retain the ability to recognize and bind molecules from a buffer solution. In fact, these surfaces show kinetic and thermodynamic binding properties for selected analyte molecules that are comparable to immobilized monoclonal antibodies, the gold standard receptors for biosensing. How can LPNE and virus particles be integrated? One approach, to be discussed in this presentation, involves the fabrication of nanowires of the conductive polymer PEDOT (poly(3,4-ethylenedioxythiophene)) in which virus particles are entrained. These composite nanowires show a change in their dc electrical resistance upon exposure to peptides that selectively bind to the entrained virus particles.

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## **Workshops**

## **EVOLUTION OF HPLC MATERIAL TECHNOLOGY: FROM IRREGULAR TO CORE SHELL SILICA**

Andrea Gheduzzi

Phenomenex srl

Silica gel is the most widely used material for normal and reversed phase since the early beginning of LC when irregular particles were packed at medium-low pressure, up to slurry packed columns using particles with a diameter even smaller than 5 $\mu$ m, thus giving rise to the well known HPLC technology(1). More recently the introduction of particles smaller than 2 $\mu$ m has opened a new era of Ultra High performance or UHPLC, very demanding in terms of 'ultra high' pressure as well, tough..

This evolution in LC particle technology has been pushed by different needs coming from main application areas. The most important is certainly the request for shorter analysis time, allowing for runs of less than 1 minute, compared to typical 30-40 minutes. This was the main goal for HPLC for Pharma companies, initially from Drug Discovery groups but currently expanding to QC labs as well.

Another very strong request is the need for better sensitivity in environmental and food safety screenings.

In Food and Environmental labs, a continuously growing diffusion of mass spectrometers coupled to LC's has made the possibility of executing multitarget analysis on broad libraries become true, similarly as it happened decades ago when GC capillary WCOT columns were introduced.

The development of faster MS analyzers and more optimized interface designs has boosted the research in new column technologies yielding sharper and more symmetrical peaks thus getting to higher S/N ratios, so important for quantitative analysis at the demanding LLOQ's levels of today's EU legislation.

For those research labs involved in the so called 'omics' the most important which is improving as much as possible the resolution in tough peptide mapping analysis and getting more details about post translational modifications or minor differences in primary structure for intact proteins.

The answer to all of these different needs is today best given by non-fully porous silica, rather than sub-2 $\mu$ m fully porous particles.

The advantages of non-fully porous particles were already known in the '60's (2), but packing materials manufactured at that age were suffering from strong limitations in retention and sample capacity. A sharp improvement was obtained about 10 years ago with the porous shell technology developed by J.J. Kirkland (3). More recently, in 2009, Phenomenex has introduced a novel proprietary Core Shell technology based on nanostructures, that was able to achieve even better results, such as achieving even higher efficiency and keeping it at linear velocities even 5 times higher than the optimal value and sharper peaks also for molecules having a very low diffusion coefficients, like peptides with Mw's above 5 KDa (4-5).

At present Core Shell represents the state of the art in UHPLC/HPLC column technology. The optimization of the ratio between the porous layer and the particle diameter allows to reach efficiency levels that range from 210,000 for the 3.6  $\mu$ m to 320,000 p/m for the 1.7  $\mu$ m, with retention and sample capacity features equivalent to traditional fully porous particles, within analytical scale.



Unlike sub-2 $\mu\text{m}$  materials, capable of Ultra High performances at ‘ultra’ high pressure only, these last generation core shell materials can deliver Ultra High Efficiency at pressure values still compatible with conventional LC platforms. Limiting the chromatographic process to the outside shell, it is possible to increase the overall particle size. This explains why a 2,6 $\mu\text{m}$  particle is able to provide the same 280.000 p/m of a 1,7 $\mu\text{m}$  fully porous particle at a 40% less pressure.

The improvement in column permeability allows to exploit this novel Core shell technology not only on newest UHPL systems but also on conventional LC platforms applying just minor inexpensive adjustments and choosing a compatible column format. At nearly no cost it is actually possible to turn a regular HPLC into a UHPLC like system.

Joining the newest Core Shell technology from Phenomenex to the low dispersion properties of newest UHPLC platforms allow to further expand the column choice and take full advantage of the Ultra High efficiency at lower pressure, developing the fastest and highest resolution separations.

#### References:

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- (2) Cs.G. Horvath, B.A. Preiss, and S.R. Lipsky, Anal.Chem. 39, 1422 (1967)
- (3) J. J. Kirkland, J. J. DeStefano, J. Chromatogr. Sci., 8 (1970) 309
- (4) F. Gritti et al., Journal of Chromatography A, 1217 (2010) 1589–1603
- (5) F. Gritti, G. Guiochon, J. Chromatogr. A 1217 (2010) 1604–1615

## **FAST GC-MS/MS ANALYSIS OF PESTICIDES (QUECHERS) USING RAPID FULL SCAN/MRM SWITCHING MODES**

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The determination of pesticides residues in food prepared by the well established QuEChERS preparation has been done by single quadrupole GCMS in selected ion monitoring (SIM) or SCAN modes, GCMS/MS using multi reaction mechanisms (MRM) and LCMS/MS (MRM).

Using single quadrupole GCMS full scan modes provide maximum safety to avoid false positive or false negative identification as matrix signals can interfere with the signals of the target compounds. Here large volume injections are required to obtain the sensitivity required [1]. On the other hand SIM in GCMS shows significant higher sensitivity for quantitative analysis but contain in presence of matrix some uncertainty for identification. Despite the fact that MRM modes in triple quadrupole GCMS/MS show certainly better safety in identification of target compounds full scan spectra search gives an additional safety check to avoid false positive or false negative identification in complex matrices. Therefore here MRM/SCAN modes were used to obtain both data sets for each pesticide in one analytical run.

In the past fast GC and GCMS using narrow bore columns have become a powerful tool to increase analysis efficiency in different fields. This approach reduces analysis time drastically while mainly maintaining the chromatographic resolution. As sample capacity for 0.1 mm inner diameter columns is reduced in comparison with standard columns in this work a RTX-5 15m, 0.15 mm, 0.15  $\mu$ m was used which was compatible with the matrices analysed. Regarding the detector part the system must be able to follow sharp increases of signals as the peak widths at half height (FWHM) in fast GC with narrow bore columns are expected to be down to about 0.5 s.

Therefore in fast GC the mass spectrometric detector must supply enough data points in order to allow quantification. Here the Shimadzu GCMS-TQ8030 triple quadrupole MS was used. This instrument offers full scan scanning speed up to 20000 amu/sec and 600 MRM transitions sequentially with a minimum dwell time of 1 msec.

The method was adapted to QuEChERS extracts of different vegetables. The limit of quantification (MRM) was below 0.1 ppB.

Its unique fast scanning rate allows to work in GC x GCMS/MS offering unique performances in simultaneous Scan/MRM acquisition in qualitative and quantitative analysis.

[1] K. Friedrich, H.D. Winkeler and H.-U Baier, The Column, March 2009

## **NEW DEVELOPMENT IN SHIMADZU TANDEM MASS SPECTROMETRY AND APPLICATION IN RESEARCH OF MYCOTOXINS IN FOOD.**

S.Zaza

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In the context of quantitative research of various substances of interest in different matrix, liquid chromatography and tandem mass spectrometry is increasing its importance due to the continuous development both in the construction of kit for the extraction of these substances and in the scientific instrumentation. Recently Shimadzu has introduced two new tools that extend its range in the tandem mass spectrometry: the new LCMS-8040 and the new LCMS-8080, that join the LCMS-8030. The new instrumentation allows to raise the limits of sensitivity; in particular the new LCMS-8040 retains the unique characteristics of LCMS-8030 in terms of absolute speed in analysis but also the new system allows to increase the sensitivity about five times respect with the LCMS-8030. The LCMS-8080 system is, instead, the top for what concerns the world of Shimadzu tandem mass spectrometry, in fact achieved a sensitivity of about thirty times higher than the first system LCMS-8030.

There are several applications for the tandem mass spectrometry, but recently one of particular interest appear to be the research of mycotoxins. Mycotoxins are fungal poisons that threaten the world food, in particular cereals and nuts. Regulatory agencies have imposed limits on levels of mycotoxins allowed in food. Food safety is ensured by testing for the presence of mycotoxins by methods such as LCMSMS. In addition to mycotoxins, many other known and unknown chemicals threaten the food supply, however their presence might be missed by LCMSMS methods, which rely exclusively on MRM settings for detection. Therefore we have developed an extremely fast and reliable method for the detection of mycotoxins in food, which has the additional advantage of collecting survey scan and data dependent MSMS for untargeted screening for other chemical threats. The method is based on a UHPLC separation performed using the Shimadzu Nexera system with the Shim-pack XR-ODS column and applying an isocratic elution using ammonium acetate water and methanol. The rapid MRM measurement was carried out on a Shimadzu LCMS-8030 triple quadrupole mass spectrometer using positive electrospray ionization (ESI).

## **PUSH THE LIMITS IN MASS SPECTROMETRY**

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The aim of the work is to highlight how it is important to push the limits in Mass Spectrometry not only for Sensitivity but also for Specificity and Selectivity. The increase of Sensitivity it is possible after the ASMS 2012 due to the introduction of a new concept of technology called “Ion Drive Technology”. The implementation of the Specificity is a consolidation of the QTRAP technology and its MRM<sup>3</sup> that is very appreciated in some quantitative experiments where the MRM does not have enough specificity for example due to the matrix effect. The Selectivity is one of the most important feature of a Mass Spectrometry and for this reason a lot of efforts are done from the R&D department in this direction and two years ago AB SCIEX produced a way to increase the Selectivity called Differential Mass Spectrometry or SelexIon.

## **Keynotes**

## SENSING SYSTEMS: STATE-OF-THE-ART AND PERSPECTIVES

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*Sensor* is one of the words that, in the last years, are most often met with in the scientific and non-scientific literature. This notwithstanding, the distinction between 'sensing system' and 'analytical instrument' is not so evident, even if nobody thinks at defining an NMR or a TEM apparatus as 'a sensor'. The feeling is that the term 'sensor' is reserved to something 'small' and performing 'punctual' detection, which are not satisfactory constraints at all.

In any cases, letting this 'semantic problem' aside, it is well known that the category to which a sensor is assumed to belong depends on the nature of the transduction. Within this frame, the most meaningful sensors in chemistry seem to be the i) electrochemical, i.e. amperometric, potentiometric, conductimetric, FET systems; ii) optical sensors, operating in transmission, absorption, or reflection, surface plasmon resonance, luminescence and fluorescence, eventually embedding the optical path into a waveguide once punctual measurements are requested and whenever feasible; iii) (micro)gravimetric sensors; iv) thermal sensing systems – the trivial thermometer or a thermocouple systems seemingly being more properly 'sensors' than calorimeter.

Hyphenated sensing systems, in which one technique generates the stimulus whose consequences are measured by a different technique – spectroelectrochemistry, electrogenerated chemiluminescence, electromicrogravimetry, thermogravimetry, etc. - are extraordinarily important and possessing potentialities still to be fully exploited.

My quick overview will deal essentially with amperometric sensing. I will consider modified electrodes, including organic modifiers, such as redox but, especially, intrinsically conducting polymers, in particular polythiophenes (PT) and inorganic modifiers, such as metal nanoparticles (NP). Special emphasis will be given to composites in which NPs will be the fillers of the organic PT matrix. The techniques for the characterisation of these complex systems will be shortly considered

The still open issues for achieving best efficiency in enzyme biosensing, including the activation of electrocatalytic oxidation of the enzyme and anchoring of the whole system on the electrode surface, will be considered. The importance of nanostructured substrates in gaining highest sensitivity in genosensors will be also stressed.

The criticisms of their the development and of those based on amperometry, will be discussed.

## **CAPILLARY ELECTROPHOREIS AND HPLC OF BIOMOLECULES: FUNDAMENTAL AND PRACTICAL ASPECTS RELATED TO THE OPTIMIZATION OF THEIR SEPARATION PERFORMANCE**

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Most chromatographic and electrophoretic separations of biomolecules are performed in aqueous solutions whose composition is one of the main factors influencing separation performance and selectivity. This aspect is particularly relevant for peptides, proteins and for a significant number of hydro soluble biomolecules bearing different functionalities consisting of hydrogen-bonding regions, hydrophobic patches and hydrophilic moieties. Such multifunctional molecules may interact to different extents with the various components of the surrounding aqueous solution and with either the stationary phase or the capillary wall, in chromatography and in capillary electrophoresis, respectively. In both techniques the prediction of these interactions and the capability of controlling their influence on the separation mechanism are of paramount importance. Capillary zone electrophoresis (CZE) is a valid tool to investigate several aspects of chemistry in solution involved in the mechanisms bringing about separations either in chromatography or in electrophoresis.

This communication discusses the results of our recent studies conducted to investigate a variety of factors that influence both electrophoretic and chromatographic behaviour of an array of biomolecules. The presentation evaluates the influence of the composition of either the electrolyte solution (BGE) or the mobile phase on the selective separation of representative biomolecules in CZE and in RP-HPLC, respectively. Appropriate selection of the composition of either the BGE or the mobile phase involves the evaluation of the equilibrium in solution that might take place between the analytes and the components of such solutions. The ionogenic nature of most of the considered biomolecules requires the control of pH, which is performed using suitable buffering agents incorporated into the BGE or the mobile phase employed in CZE and in HPLC, respectively. The constituents of the buffer solutions do not limit their action at controlling the protonic equilibrium in solution of the analytes bearing ionogenic groups. They may also interact with the biomolecules, for examples by an ion-pairing mechanism, with the result of altering either the electrophoretic mobility or the chromatographic retention of these analytes, resulting in significant variations of selectivity. The result is the possibility of tailoring selectivity and efficiency of the considered separation systems by incorporating suitable buffering agents and additives into the BGE or the mobile phase, respectively. Practical applications of these approaches to the analysis of biomolecules in complex matrices are then discussed.

## **NEW PERSPECTIVES IN CHEMOMETRICS**

R. Todeschini

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This talk will present some new trends and ideas to overcome the prevailing issues within chemometrics, especially with Exploratory Data Analysis. A simple approach based on a new covariance matrix will be discussed explaining how two matrix parameters can allow an efficient way to detect two different set of outliers. Moreover, it is very clear that the chemometricians are currently more involved in dealing with complex systems, however, the classical approaches such as Principal Component Analysis, cluster analysis and multidimensional scaling are somehow limited to reflect only the linear relationships. To overcome this major limitation, a non-linear data exploration based approach namely 'Atemporal Target Diffusion Model (ATDM)' was recently proposed. A brief overview of this new approach will be also presented along with some case studies.



## **THE CHEMISTRY OF EQUILIBRIA IN SOLUTION AND RELATIVE SPECIATION STUDIES: PAST, PRESENT AND FUTURE**

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In the last century many studies were performed on solution chemistry equilibria, using, in particular, potentiometric, spectroscopic and polarographic techniques. Up to 1960 the main aim of these studies was the determination of equilibrium constants. Shortly afterwards, many other techniques have been employed (being NMR the most used) and the solution studies were devoted to a new topic called Speciation (term borrowed from biology). Pioneering works on Speciation were the computer assisted equilibrium analysis of oceans (L.G.Sillen, 1967) and blood plasma (Agarwal & Perrin, 1976). Nowadays, the concepts of Equilibria in Solution and Speciation are strictly bound. Moreover, these studies are often performed in different ionic medium conditions (to simulate different natural and biological fluids), and, in addition to the formation constants, enthalpy and entropy contributions are determined by temperature gradients or by titration calorimetry. Future developments include a) the improvement of experimental and calculation techniques; b) improving databases containing also critical values of formation thermodynamic parameters; c) modeling the stability, the enthalpy changes, the activity coefficients, the solubility, and so on, necessary for the speciation of particular systems. Items a) and b) do not need particular explanations. Experimental and calculation techniques are continuously improving and some coupled instrumentations (for example, potentiometric –spectrophotometric or calorimetric – potentiometric) are particularly promising in the accurate study of multiple equilibria. Item c) is, in my opinion, the most interesting. Why? The number of possible interactions between cations and anions certainly exceed the possibility of experimental determination in a short period. Therefore we need some modeling tools for predicting formation thermodynamic parameters. How? There are many correlations among different parameters for different metals and ligand classes and, using critically the available databases and building suitable models, it is possible to reduce considerably the number of experiments to be performed. Recently, the modeling of Cadmium(II) interactions with different classes of ligands, has been proposed (1).

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## **DEVELOPMENT OF ANALYTICAL METHODS FOR THE CHARACTERIZATION OF POTENTIALLY HEALTH BENEFITING FOODS AND FOOD INGREDIENTS**

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In the last two-three decades consumer demands in the field of food production has changed considerably and foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but also to prevent nutrition-related diseases and improve physical and mental well-being. In this regard, functional foods play an outstanding role. The increasing demand on such foods can be explained by the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for improved the quality their life. the term “*functional food*” itself was first used in Japan, in the 1980s, for food products fortified with special constituents that possess advantageous physiological effects. The three basic requirements to be considered as a functional food include 1) derived from a natural occurring ingredients; 2) consume as a part of daily diet; and 3) involve in regulating specific process for human including delaying aging process, preventing the risk of disease and improving immunological ability.

So far a large number of functional foods in various forms have already been introduced into the market. Many of them contain a number of characteristic functional ingredients. They include dietary fibre, oligosaccharides, sugar alcohols, peptides and proteins, prebiotics and probiotics, phytochemicals and antioxidants, as well as polyunsaturated fatty acids

However, in order to achieve adequate regulatory control, an explicit definition of functional foods is crucial. Common terminology must be the basic requirement on discussing the relating issues among academy, industry and government.

Furthermore, advanced analytical methods are usually needed for the characterization of functional foods and functional ingredients, their control during food processing and storage, the characterization of original bioactive compound or their metabolites. This presentation deals with the development of analytical methods to characterize prebiotic carbohydrates as well as phenolic compounds in food and food by products.

## GREEN CHEMISTRY: AN ANALYTICAL PERSPECTIVE

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In the last two centuries chemistry has improved our quality of life through the production of thousands of useful products and materials, but this achievement comes at an environmental price. Green chemistry and its principle want to reduce or eliminate the negative environmental impacts and through design, innovation and new processes to restore the planet's sustainable development. The term "Green Analytical Chemistry", proposed by J. Namieśnik [1] in the early 2000, at the beginning was scarcely employed in the analytical chemistry community in contrast with green catalyst development and green organic chemistry concepts. Nonetheless, efforts made in analytical chemistry in the past 10 years have led to the adaptation of existing methods and development of new techniques to save time and chemicals, and to improve overall performance in agreement with the green chemistry principles. It seems straightforward to consider green analytical chemistry as that part of the green chemistry devoted to analysis. The impact of the application of green principles to analytical chemistry can be easily realized by considering the number of analysis required around the world to control our health, the quality and safety of all kinds of products and to monitor the environment. In fact, it is well known that analysis requires employment of a great amount of chemicals and energy and it provides some collateral risks for both, operators and the environment, due to the use of toxic reagents and solvents and the generation of dangerous wastes. In this keynote lecture, some of the main tools to greening analytical procedures will be revised. In particular, the talk will focus on the efforts required for improving the analytical practices in order to minimize adverse effects such as: the replacement of toxic solvents and reagents by safer ones, the miniaturization of analytical procedures with a resulting reduction in the waste production and analysis time, the strong reduction of the analytical steps or the analysis of untreated samples. All of these steps are significant parts of methodologies that could contribute to improve the safety of analytical procedures and to minimize environmental dangers. Moreover, as green analytical chemistry procedures have to maintain and/or improve the quality of analytical data, chemometric aspects will be examined. In particular multiparametric measurements and remote sensing methodology able to enhance the information obtained with reduced analytical task will be discussed.

[1] J. NAMIESNIK Green analytical chemistry - some remarks. *J. Sep. Sci.* 24,10, 2001.

## **NEW INSIGHTS ON THE INTERACTIONS BETWEEN ENVIRONMENT AND CULTURAL HERITAGE**

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Ancient architecture, built environment and artwork collections attract millions of tourists every year to historical cities and archaeological sites, museums, libraries, etc.. To take care of such a richness –both indoors and outdoors – is actually a challenge. In particular, environmental change – further complicated by the increasing influence of climate change – presents one significant threat to the sustainability of the cultural heritage

The lecture will focus on the update description of urban atmospheric pollutants and their impact assessment, inviting to discuss on what it actually means the conservation of cultural heritage. Extreme events such as earthquakes together with non-extreme events (climate change, environmental pollution) will also be considered.

For example, our historical cities are subjected to modified environmental conditions, characterized by a lower concentration of pollutants which were usually taken into account (SO<sub>x</sub>, NO<sub>x</sub>, particulate) and by an increase of VOCs: thus, artworks will be affected in a different way, and they will require a different conservative approach. Atmospheric pollutants may have deep oxidative effects on the conservation of paintings in Museums causing color fading, detachments, yellowing: are the microclimate frames - nowadays so widespread- the proper tools for a better storage?

The continuous interaction of cultural heritage objects with the external environment necessitates an approach based on prevention, management and maintenance: particularly, to preserve the environment is a must to slow down the actual decay rate of cultural heritage.

## KEY ISSUES IN MODERN ANALYTICAL TOXICOLOGY

M. Vincenti<sup>1,2</sup>

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The screening of psychoactive substances in biological specimen may have a variety of objectives, in terms of both target chemical classes and purposes of control, but the legislation of most Countries make a clear distinction between drugs of abuse, whose use is prohibited in almost all circumstances, and pharmaceutical substances, whose use is permitted under medical prescription, although they are occasionally abused. The detection of the drugs of abuse in biological samples is increasingly requested for the withdrawal control of habitual drug abusers and in other toxicological investigations, such as workplace drug testing driving re-licensing, pre-natal exposure to drugs and post-mortem toxicology. All these screenings basically require sensitive detection of relatively few target compounds.

However, it is quite frequent that the target analytes cannot be foreseen, so that a wide range or "general" screening of drugs and venoms is commonly required. This is the case of most acute intoxications and autoptic investigations. Whenever a multitude of candidate substances may represent the cause of intoxication or death, several analytical procedures are likely to be utilized on the collected biological specimen, with direct impact on time, costs and efficiency. For this reason, comprehensive screening procedures of multi-class drugs are progressively introduced into the analytical practice. A similar situation applies to drug-facilitated sexual assaults, when a variety of psychoactive drugs may have been used to alter the victim's degree of awareness and memory, including benzodiazepines, hypnotics, sedatives, anesthetics and drugs of abuse. The forensic investigation is addressed to both traditional matrices, such as blood and urine, as well as alternative matrices, including hair, oral fluid and sweat, in order to cover periods of time after drug intake ranging from immediate to several months.

The recent major improvements of chromatography and mass spectrometry (MS) have driven analytical toxicology toward previously inconceivable results. Among these, it is impressive to note that segmental hair analysis can nowadays provide evidence of a single administration of drug, which occurred several months before the analysis. The continuous enhancement of MS sensitivity is progressively reducing the amount of sample needed for carrying out the chemical analysis, extending the chance of investigation to newborn children, for the detection of prenatal exposure to drugs.

Another key issue of toxicology is the detection of new synthetic drugs, that are continuously introduced into the black market. For these substances, the metabolism is frequently not known and pure standard are missing, making the development of adequate analytical methods difficult.

## WHERE IS AND WHERE IS GOING FORENSIC ANALYTICAL CHEMISTRY

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Innovations in Forensic Analytical Chemistry mainly concern the applications of the analytical improvements to matrices of forensic interest.

The great deal of sensors of several types has been applied to detections of compounds of forensic interest, like pigments and dyes, drugs and explosives for the homeland security. The identification of explosives after the blast has also made possible by sensors and biosensors<sup>1</sup>. In fact analysis with sensors is usually cheap, fast and can often be performed in situ.

On the other hand some work still needs to be made to improve the reliability and durability of sensors<sup>2</sup>. Nevertheless a patent is pending for a very cheap multichannel sensor able to identify both explosives and drugs that can be mounted in a cellular.

The huge progress in Raman instrumentation also allows for rapid detection and identifications of drug and explosives on solid surfaces<sup>3</sup>.

Recent progresses in SPME technology also gave a strong push in lowering detection limits in vapors of illicit substances<sup>4</sup>.

Thanks to important improves in HPLC-MS techniques, toxicology could undertake studies in the new fields Marco Vincenti will talking about.

Combined Raman and HPLC-MS has been able to identify proteins, and then matrices (blood, saliva, sperm) on the same very small samples on which DNA has been individuated.

From a more methodological point of view, the courts are more and more accepting the point that the absolute certainty doesn't belong to this world. So forensic scientists more and more are using statistics, both for dealing with the analytical results, and for giving the judges the probability of accuracy of their conclusions (error ratio)<sup>5</sup>. So the quality assurance is at last taking off in public forensic institutions. Unfortunately private labs still dislike QC, and this is still a serious risk of damaging the enforcement of criminal law.

Chemometric techniques, like discriminant analysis and multivariate calibration, is getting popular among forensic chemists. However too often the validation and the discussion of the proposed models is lacking, so that chemometrics seems just to be used as an ornament of the performed analyses.

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## **BIOANALYTICA, BIOSENSORS, AND WELL BEING**

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In recent years the progress of bioanalytical chemistry has grown exponentially.

Proteomics, metabolomics and DNA biochips coupled with modern and sophisticated instrumentation as mass spectrometry, fluorescence and chemiluminescence, imaging and NMR have brought new solutions in the area of medicine, for early diagnosis of major diseases, in food safety and control, also in the detection of pollutants in the environment at very low concentrations.

One of the most strategic areas of the bioanalytical chemistry is the development of biosensing technologies and biochemical sensors as one class of the bioanalytical tools used for screening methods in clinical, food and environmental analysis.

This presentation will cover the development of electrochemical biosensors and immunosensors and their applications in medicine such as the measurement of transcription factors in cell extract using DNA sensors, the direct detection of the hepatitis A virus using an immunosensor, the detection of anti-transglutaminase antibodies in blood using immunomagnetic beads and SPE-arrays for the diagnosis of celiac disease. Attempts of their detection in saliva will also be shown.

Some applications in food analysis as the detection of palitoxin in waters using screen printed electrodes coupled with suitable mediators, the detection of lead in milk, and the control of alcoholic fermentation of musts by monitoring wine key metabolites will be presented.

Concerning the environment, a prototype based on enzyme inhibition has been developed and applied for the detection of nerve agents in the air.

In the area of nanotechnology and biomedicine, Ionic Liquids (ILs) were used for the exfoliation of multi-layers of Graphene Oxide (GO) or Reduced Graphene (GR) nanoribbons to assemble a new class of modified electrochemical sensors for heparin detection. These nanocomposites GO/ILs and GR nanoribbons/ILs could be also applied for a quantitative measurement of the human serum chorionic gonadotropin (hCG).

## **NANOANALYTICS : ANALYSIS OF “NANO-MATERIALS” AND “NANO-MATERIALS” FOR ANALYSIS. STATE OF ART AND PERSPECTIVES.**

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Over the last 15-20 years, nanostructured materials have revolutionized Analytical Chemistry discipline. The term *nanoanalytics* actually labels two distinct, though largely inter-connected, fields which have experienced a considerable growth both from a fundamental and from an application point of view.

As a matter of fact, from one end the development of synthetic strategies based on either a bottom-up or a top-down approach for the preparation of nanomaterials of different shape and morphology needs a validation of the synthesis effectiveness by the use of techniques able to characterize newly synthesized nano- samples. Size, shape, chemical composition, optical properties need to be evaluated and these investigations may be particularly intriguing when nano-structured materials are surface modified with complex architectures in order to obtain probes with specific targeting.

From the other hand, nano-materials have rapidly moved into the mainstream for chemical and biological analysis. Nanoparticles, in particular, have been widely applied for sensing, imaging, catalysis, electronics, optics and optoelectronics. The use of nanostructures made possible to probe biological materials with higher sensitivity or higher specificity at nanoscale. Moreover, some well-established analytical techniques have been revitalized by the introduction of nano-structured material. Surface Enhanced Raman Spectroscopy (SERS), Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS), and Fluorescence are important examples.

In Raman spectroscopy the surface enhanced effect obtained by the use of nanoparticle substrates leads to combine molecular fingerprint specificity with potential single-molecule sensitivity(1). Introduction of nanoparticles into mass spectrometric research greatly influenced the application of this technique into various “-omics” and microorganisms detection (2). In fluorescence measurements, composite nanoparticles present substantial signal amplification and improved multiplexing for higher sensitivity and resolution, moreover, these multifunctional nano-materials represent a bright future for quantitative measurements (3).

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## **ANALYTICAL SPECTROSCOPY: STATE OF THE ART AND FUTURE PERSPECTIVES**

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Spectroscopic methods have played a fundamental role in the development of chemistry and still maintain a similar role even in modern chemistry. In particular, analytical chemistry continuously benefits from the powerful support of both standard and even more innovative spectroscopic methods. These methods help in providing a detailed chemical description of complex matrices ranging from polymeric systems to nanostructured materials and colloids. Surface chemistry has also greatly benefited from the use of spectroscopic analytical methods.

Over the last decade great emphasis has also been given to spectroscopic methods useful to study biochemical systems. In this perspective, an important role is played by spectroscopic methods exploiting special enhancement effects such as that given by localized surface plasmon resonance.

## **DIRECT-EI LC-MS INTERFACE: CAN LIQUID CHROMATOGRAPHY AND ELECTRON IONIZATION MASS SPECTROMETRY WORK TOGETHER AGAIN?**

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Electrospray Ionization (ESI) has become a de facto standard methodology for interfacing HPLC with MS. However, many shortcomings of ESI are overlooked or neglected especially when complex matrices are involved. Ion suppression limits reproducibility and accuracy. Cappiello et al. have been pioneering the efficient direct coupling of capillary HPLC with EI-MS and have recently demonstrated feasibility of working in the nanoflow-HPLC coupling with GC-MS type detectors. The technique has proven its effectiveness for a wide range of compounds. In this presentation, the authors want to give an overview about the principle methodology, figures of merit and up-to-date validation and application studies executed. Special focus will be given on new high-temperature applications. The interfacing mechanism is based on direct introduction of a liquid phase into the EI source followed by complete conversion to the gas phase prior to a conventional, electron-assisted ionization (1, 2). The mass spectrometer is an Agilent 5975B Inert MSD, a single quadrupole instrument coupled to an Agilent 1100 Series nano-HPLC system. The transition mechanism is based on the formation of an aerosol in high-vacuum conditions, followed by vaporization of the solute upon contact on the hot surface. Nano liquid chromatography was performed in reversed and normal phase conditions using a mixture of water, acetonitrile or hexane and isopropanol respectively. 75  $\mu\text{m}$  i.d. columns, packed with C18 particles were used for all RP separations. The new, EI-based interface shows no matrix effect under many experimental conditions and allows an alternative LC-MS detection for innovative fields of application. A new nebulizer that can operate at high-temperature (up to 350°C) is presented. It is machined from a full metal block that holds the transfer capillary in place and applies the heat, only during nebulization, directly at the tip of the transfer capillary. A better vaporization of the analytes, without a premature mobile phase vaporization inside the capillary is obtained. The higher temperature extends the number of amenable compounds and the range of possible applications.

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## **Comunicazioni orali**

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

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

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

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

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In the last years a wide variety of electrode systems, especially based on nanostructured materials was explored, aiming at improving the analytical performance of electrochemical sensors in terms of sensitivity, selectivity, stability, etc. Recently the group of Tor Vergata developed electrochemical sensors based on screen printed electrodes (SPEs) modified with carbon black (CB). The CB-SPEs showed an enhanced oxidation current for several analytes when compared with bare SPEs (1-3). At the same time, the group in Modena has acquired wide expertise in the use of gold nanoparticles (AuNPs) in electroanalysis: effective electrocatalytic processes have been activated by fixing AuNPs on electrode surfaces (4,5).

In this work SPEs were modified by depositions consisting of CB and AuNP nano-composites (AuNPs-CB-SPEs). Using a fixed amount of CB to modify the SPEs (1), the amount of AuNPs was optimized. The AuNPs-CB-SPEs were characterized by morphological and electrochemical techniques, demonstrating that *i*) the CB coating was very stable fixing AuNPs; *ii*) the AuNPs fixed on the CB lead to a nanostructured, very homogeneous composite; *iii*) the nano-composite material is characterized by an enlarged electroactive surface with respect to both the single modified components. Several analytes, such as NADH and glutathione, have been tested on AuNPs-CB-SPEs, demonstrating an improvement of electrocatalytic properties when compared with SPE modified either with CB or AuNPs as the single component. A synergic effect of the two materials is evidenced. The sensor modified with AuNPs-CB nano-composite was also tested for As (III) detection, using anodic stripping voltammetry. The different experimental conditions were investigated and optimized, allowing the detection of As(III) at the legal limit level in drinking water (10 ppb).

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

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

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## **AFFINITY ELECTROCHEMICAL BIOSENSORS BASED ON NANO-ELECTRODE ENSEMBLES**

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

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

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

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

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

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

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A way to quantify the signal drift causes of an oxygen optical sensor has been developed. Theoretical formalization were experimentally confirmed by using a polysulfone-based thin layer membrane embedding platinum meso-tetra-(pentafluorophenyl)-porphyrine working in light emission detection mode. Photochemical, thermal and oxidative degradation of both luminophore and polymeric matrix were rationalized and tested. Experimentally determinable light

intensity drift,  $D_I$ , and Stern-Volmer constant ( $K'_{SV}$ ) drift,  $D_K$ , were related to matrix and luminophore modifications. The mathematical modeling of the drift

evidenced that  $D_I$ , is constant by increasing the % $O_2$  only in the absence of  $D_K$ . It

has been possible to quantify all the contributions independently through a very good match between theory and experiments. The sensor drift analysis allowed understanding whether luminophore and/or polymer degradations were operative. In the studied case oxidative degradation was absent; thermal degradation of the

sole luminophore, was independent of % $O_2$  and caused a relative light intensity

drift of  $-0.028(0.002) \text{ day}^{-1}$ ; photochemical degradation was present both on

luminophore and polymeric matrix. When  $D_K \neq 0$ , also the commercially

available oxygen optical sensors based on phase-shift and the life-time detection modes lead to incorrect oxygen concentration values and should account for the drift analysis proposed in the present study.



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

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

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

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

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

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

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

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

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

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

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

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Designing stationary phases based on the excellent adsorption properties of nanomaterials is a hard but fascinating challenge; in this context, the use of carbon nanotubes (CNTs) for preparing novel chromatographic sorbents is a current trend in analytical chemistry (1).

In the last years we have synthesized chemically-modified CNTs, which gave excellent performance as GC stationary phases, behaving as mixed-mode separation materials (2-4).

In this contribute we present the first results on the application as HPLC stationary phase of a novel material based on silica microspheres functionalized with multi-walled CNTs (MWCNTs). These were grafted by  $\gamma$  radiation onto silica microspheres in presence of polybutadiene (PB) as the linking agent. The final product (MWCNT-PB-modified silica) showed homogeneous particle size and high surface area. The chromatographic application of MWCNT-PB-modified silica gave interesting results in the separation of different classes of compounds: aromatic hydrocarbons, chloroaromatics, and a variety of substituted benzenes. Satisfactory resolution and selectivity were obtained also for closely related analytes, for instance dichlorobenzene regioisomers. Reproducibility in retention time (RSD < 2%) evidences the chemical stability of this new phase. Comparative experiments on PB-modified silica proved the key role of MWCNTs. The application to the analysis of real samples will be reported.

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

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

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## PHENOLIC ACIDS AND ANTIOXIDANT CAPACITY IN DURUM WHEAT AND ITS PRODUCTS

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

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

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

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

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

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

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

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

In this framework, aim of the present study was to investigate the possibility of using the aquaphotomic approach for the quantification of different inorganic salts in water. To this purpose, aqueous solutions of 6 salts ( $\text{AlCl}_3$ ,  $\text{KCl}$ ,  $\text{MgCl}_2$ ,  $\text{NaCl}$ ,  $\text{KNO}_3$ , and  $\text{NaNO}_3$ ) at different concentrations were prepared and analyzed. Successively, calibration models for the quantification of the studied solutes were built using Partial Least Squares regression, after variable selection by Genetic Algorithms (GA) coupled to backward interval-PLS (biPLS, 3). This variable selection procedure, together with improving the predictive ability of the models, allowed a clearer interpretation of the results, highlighting the spectral regions characteristic of the interactions with the different solutes.

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

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## MULTIVARIATE STATISTICAL OPTIMIZATION OF BIOHYDROGEN PRODUCTION FROM CRUDE GLYCEROL

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

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

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

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

In the following part of the project, Design of Experiments (DoE) was applied to improve the ability of enriched activity sludge to efficiently convert crude glycerol into hydrogen. Plackett-Burman screening design identified initial glycerol concentration, temperature and initial pH as important variables. Box-Behnken design was then used for optimization. The maximum hydrogen yield of 0.96 molH<sub>2</sub> /molglycerol was estimated at temperature 37°C, initial pH 7.9 and initial glycerol concentration 15.0 g/L. The last part of the project, that involves the scale-up and the study of the process in a continuous mode, is currently under investigation.



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

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

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

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

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

Other approaches have been suggested in this contest; for example the Principal Component Analysis (PCA) can be performed separately for each data set and the first Principal Components of each block can be considered and joined (3).

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

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

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

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

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## COUPLING 2D-WAVELET DECOMPOSITION AND MULTIVARIATE IMAGE ANALYSIS

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

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

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

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

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

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

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## BINDING ABILITY OF GLUTATHIONE TOWARDS METAL AND ORGANOMETAL CATIONS

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

In order to evaluate the sequestering ability of glutathione and, therefore, its possible use as detoxificant agent in biomedical applications or to remove toxic metal ions from natural systems, we undertook a study on the interactions between this ligand and metal ( $\text{Hg}^{2+}$ ,  $\text{Pb}^{2+}$  e  $\text{Zn}^{2+}$ ) and organometal cations [ $\text{CH}_3\text{Hg}^+$ ,  $(\text{CH}_3)_2\text{Sn}^{2+}$ ,  $(\text{CH}_3)_3\text{Sn}^+$ ,  $(\text{C}_2\text{H}_5)_3\text{Sn}^+$ ,  $(\text{C}_3\text{H}_7)_3\text{Sn}^+$ ]. For all systems, studies were performed by potentiometry at different ionic strength ( $0.1 \leq I \leq 1 \text{ mol L}^{-1}$ ) and temperature ( $15 \leq t \leq 45 \text{ }^\circ\text{C}$ ). In addition, UV-Vis and  $^1\text{H-NMR}$  spectroscopy, in order to confirm the speciation models, and titration calorimetry, in order to give a complete picture of the thermodynamic properties, were used.

Speciation models of *GSH* - $\text{Hg}^{2+}$ , - $\text{Pb}^{2+}$  and - $\text{Zn}^{2+}$  systems were quite similar with the formation of different  $\text{ML}_i\text{H}_j$  ( $i = 1,2$ ;  $j = 0,1,2$ ) species, together with  $\text{ML}(\text{OH})$  and  $\text{ML}_2(\text{OH})$ , for  $\text{Zn}^{2+}$ . The stability of species was very different: as an example, for  $\text{ML}$   $\log\beta = 32.31$ ,  $9.60$  and  $8.80$ , for  $\text{Hg}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Zn}^{2+}$ , respectively (at  $I = 0.1 \text{ mol L}^{-1}$  and  $t = 25 \text{ }^\circ\text{C}$ ). Also for *GSH*-organometal systems, very similar speciation models were obtained, with formation of  $\text{ML}$ ,  $\text{MLH}$  and  $\text{MLH}_2$  species, together with  $\text{ML}(\text{OH})$ , for  $(\text{CH}_3)_2\text{Sn}^{2+}$  only. Also in this case, the complexes with mercury(II) are more stable respect to those of other organometal cations.

On the basis of formation constants and speciation profiles, the sequestering ability of *GSH* towards different metals and organometals was quantitatively evaluated by determining an empirical parameter ( $\text{pL}_{0.5}$ ) that numerically represents the ligand concentration  $[-\log(\text{total ligand concentration})]$  necessary to sequester 0.5 of metal ion fraction. The sequestering ability of glutathione was very high towards  $\text{Hg}^{2+}$  and  $\text{CH}_3\text{Hg}^+$  and in particular experimental conditions significant also towards  $\text{Zn}^{2+}$  and  $\text{Pb}^{2+}$ .

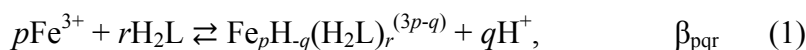
## POTENTIOMETRIC AND LASER DESORPTION MASS SPECTROMETRIC INVESTIGATION ON *trans*-HYDROXY-L-PROLINE AND Fe(III) EQUILIBRIA

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Hydroxyproline is an amino acid particularly abundant in the collagen, and its concentration in plasma and urines is considered an index of the metabolism of the collagen itself. It contains different donor atoms and for this reason it is considered a molecule with strong binding capacities (1, 2). In this work the complex formation between the Fe<sup>3+</sup> ion and *L-trans*-hydroxyproline, H<sub>2</sub>L, has been studied at 298.15 Kelvin in 0.1 mol dm<sup>-3</sup> in two different media, namely NaClO<sub>4</sub> and NaCl, in order to compare the results and to ensure that the ionic environment does not affect results.

The composition of the solutions was determined by potentiometric titrations by measuring with a glass electrode the competition of the *L-trans*-hydroxyproline for the metal and H<sup>+</sup> ions. The concentrations of ligand (C<sub>L</sub>) and Fe<sup>3+</sup> (C<sub>M</sub>) were varied between (1·10<sup>-3</sup> and 10·10<sup>-3</sup>) mol dm<sup>-3</sup>, and the ligand-to-metal ratio was varied between 1 and 10 (1 ≤ C<sub>L</sub>/C<sub>M</sub> ≤ 10). The hydrogen ion concentration was varied from 1·10<sup>-3</sup> mol dm<sup>-3</sup> to incipient precipitation of basic salts which takes place in the range [H<sup>+</sup>] = 1·10<sup>-4.5</sup> – 1·10<sup>-5</sup> mol dm<sup>-3</sup> depending on the specific ligand to metal ratio.

The general equilibrium can be written, schematically, for all systems as equation 1:



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

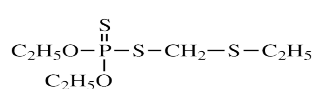
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## ADSORPTION OF PHOSPHOROTHIATES PESTICIDES ONTO AMORPHOUS IRON (III) PHOSPHATE

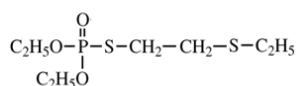
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The widespread use of organochlorine pesticides in agriculture poses the problem of their distribution in the environment. Adsorption is one of the main mechanisms of retention of pollutants. Widely used pesticides are phosphorothioates aliphatic as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



(A)



(B)

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

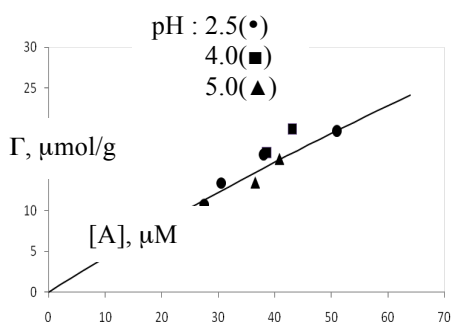


Figure 1

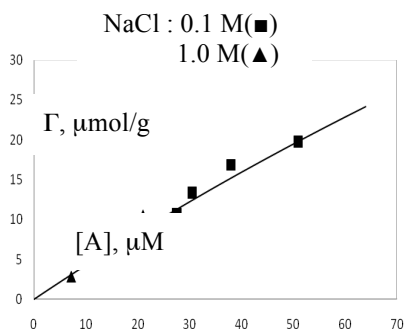


Figure 2

[<sup>1</sup>] Tiessen, H. 1995. *Phosphorus in the global environment: transfers, cycles and management*. Chichester. J. Wiley and Sons, Inc. New York

[<sup>2</sup>] W. Strumm, J. J. Morgan, *Aquatic chemistry*, third edition, 1996, John Wiley & Sons, Inc. New York



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

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Aim of this study was to investigate the quali/quantitative profile by IC (Ion Chromatography) technique of the inorganic constituents (cations:  $\text{Na}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{NH}_4^+$  and anions:  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ ) and compare the results obtained from honeys of different Italian Regions (Lombardy, Piedmont, Sardinia, Calabria, Tuscany) with those from countries of the Western Balkan area (Slovenia, Croatia, Serbia, Kosovo, Macedonia and Albania). The mineral concentrations of the two honeys groups were further analyzed by multivariate statistical techniques such as principal component analysis (PCA) and hierarchical cluster analysis (HCA). The results provide a detailed and exhaustive view of the ionic composition of the different honeys, up to now never reported. They can be applied for the detection of differences in mineral concentrations among honeys allowing, from a botanical point of view, a sharp differentiation between nectar honeys and arboreal/honeydew honeys (discrimination of the floral source) and used as an index of the purity of the matrix or of its fraudulent adulteration with sugars, syrups, etc.. Finally the obtained results can be used to individuate the natural (by bees) or artificial blending between different honeys. Furthermore the multivariate analysis allows to demonstrate the potential of honey as bioindicator of the distribution of impact of various environmental pollutants of industrial and urban origin ( $\text{Br}^-$ ,  $\text{SO}_4^{2-}$  and  $\text{PO}_4^{3-}$  contents) which show a steep increase in honeys of Western Balkan area. In particular for what concerns arboreal honeys the concentrations of  $\text{SO}_4^{2-}$  and of  $\text{PO}_4^{3-}$  in honeys from Balkan area were three times greater than those present in the Italian ones ( $\text{SO}_4^{2-}=90.3\pm 60.3$  ppm vs.  $\text{SO}_4^{2-}=29.6\pm 20.8$  ppm;  $\text{PO}_4^{3-}=772.6\pm 530.3$  ppm vs.  $\text{PO}_4^{3-}=222.0\pm 74.2$  ppm respectively).  $\text{Br}^-$  was undetectable in almost all the Italian honeys and its presence in Balkan honeys could be attributed the use of methyl-bromide for agricultural use. The findings reported in this presentation fit into a research program that aims to typify the quality of honeys in different countries.

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

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

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

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

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

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

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## PRELIMINARY STUDY ON QUANTIFICATION OF $\alpha_{s1}$ -CASEIN VARIANTS IN GIRGENTANA GOAT BREED BY DIRECT CHROMATOGRAPHIC ANALYSIS OF MILK

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Goat  $\alpha_{s1}$ -casein is a highly polymorphic protein, coded by *CSN1S1* gene. Nowadays, several alleles were identified and associated with different levels of  $\alpha_{s1}$ -casein in goat milk. Polymorphisms at  $\alpha_{s1}$ -casein locus have been shown to affect not only the quantity of this casein in goat milk, but also the structural and nutritional characteristics (hypoallergenic properties) and technological properties of the milk (1). The aim of this work was to separate and quantify the most common allelic variants of  $\alpha_{s1}$ -casein in milk of Girgentana goat breed, a Sicilian autochthonous breed, and to evaluate the effect of  $\alpha_{s1}$ -casein polymorphisms on casein content.

The *CSN1S1* A/01, B/E, F, and N alleles were simultaneously investigated by PCR-RFLP (2). AS-PCR was used for the detection of the *CSN1S1* E (3) and 01 alleles (4). Milk samples were prepared following the method proposed by Bobe et al. (5) and analyzed by RP-HPLC method (6). A reversed-phase analytical column C8 (Zorbax 300SB-C8 RP, 3.5 $\mu$ m, 300 $\text{Å}$ , 150 $\times$ 4.6 I.D.) was used and the detection was made at a wavelength of 214 nm. The procedure was developed using individual raw milk samples of Girgentana goats. For calibration experiments, pure genetic variants were extracted from individual milk samples of animals with known genotypes, considering that commercial standards for goat allelic variants were not available. In particular, were used animals with AA, BB, FF and NN homozygous genotypes. Method validation consisted in testing linearity, repeatability, reproducibility and accuracy. A linear relationship between the concentrations of proteins and peak areas was observed over the concentration range, with low detection limits. Repeatability and reproducibility were satisfactory for both retention times and peak areas.

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### Acknowledgements

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

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

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## THE EVOLUTION OF ENVIRONMENTAL ETHICS FOR A SUSTAINABLE WORLD

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

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

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

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

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

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

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

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

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



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

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The possible market for photocatalytic materials, in the form of powders, built-in powders, thin films and nanostructured materials, as well as devices directed to specific applications, is estimated in exponential growth (1).

The central problem for standardization of photocatalytic efficiency of whatever material is the rate evaluation. Among the used, proposed or approved protocols of standardization of gas/solid activity there are different reactor configurations (plug (PFR, Plug Flow Reactor) or continuous flow (CSTR, Continuous Stirred-Tank Reactor) as well as batch reactors or circulating fluidized bed) using different kind of substrates (VOC, NO, mixture of NO+NO<sub>2</sub>, NO<sub>2</sub>, acetaldehyde, toluene, formaldehyde, methyl mercaptan). However, depending on reactor type and conditions, the analytical measurements gives different calculated rates.

As "Analytical chemistry is the science of obtaining, processing, and communicating information about the composition and structure of matter" according to the definition of ACS (2), and because "...measurements [of analytical chemists] are used to assure compliance with environmental and other regulations", in this broad meaning the analytical chemist has to correctly measure the concentration as a function of time or the inlet and outlet concentrations in the above reactors, but also to find the right way to measure what regulation needs. In this framework, the basic equations governing the above reactors and the rate expression for them are here presented. Experiments using either the abatement of NO and toluene as model pollutants show that a CSTR configuration presents a lot of advantages for practical use, as any volume, any shape of catalyst, and any flow of gas into the reactor can possibly be used. A CSTR configuration is superior to the standardized PFR used in the ISO test 22197-1:2007, as the resistance to mass transfer, which leads to under-evaluate the rate, can be reduced by inside forced ventilation. Consequently, it gives an assessment of the photocatalytic rate more close to the actual surface one. The rate for CSTR at steady state must be calculated as  $r(C_o) = C_o F \eta / (1-\eta)$ , where  $\eta$  is the conversion. The revision of the standard methods ISO 22197-1:2007 and UNI 11247-2010 for photocatalytic materials is needed.

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(1) COST Action 540 "PHONASUM", 2007-2010, Photocatalytic Technologies and novel nanosurface materials, see <http://www.cost540.com/content/cost1.html>

(2) [http://portal.acs.org/portal/acs/corg/content?\\_nfpb=true&\\_pageLabel=PP\\_ARTICLEMAIN&node\\_id=1188&content\\_id=CTP\\_003375&use\\_sec=true&sec\\_url\\_v ar=region1&\\_\\_uuid=4a79f156-a321-4daa-bab0-129de427fb89](http://portal.acs.org/portal/acs/corg/content?_nfpb=true&_pageLabel=PP_ARTICLEMAIN&node_id=1188&content_id=CTP_003375&use_sec=true&sec_url_v ar=region1&__uuid=4a79f156-a321-4daa-bab0-129de427fb89)

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

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

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

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

## STABILITY CHARACTERISATION VIA ISOTHERMAL AND SCANNING CALORIMETRY

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

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

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

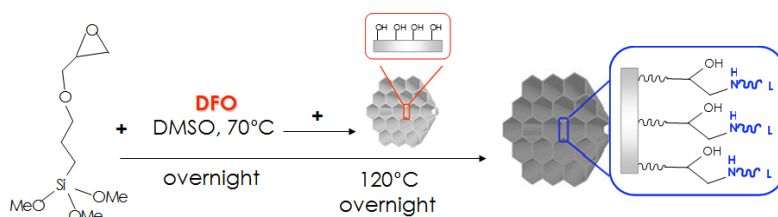
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## TOWARDS TOTAL AND FREE IRON(III) SENSING

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



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

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

In the second part of the project, we have explored the sorbing properties of the optimized product. A series of experiments in controlled  $\text{KNO}_3$  and urine media demonstrates that in a wide pH range the complex  $\text{FeHL}$  is formed between the anchored DFO and the sorbed  $\text{Fe(III)}$  ions. The exchange coefficient,  $\log\beta_{\text{ex}} = 40.1(2.5)$ , is in pretty good agreement with the property of the ligand in solution.

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

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## ADSORPTION OF SELECTED PHARMACEUTICALS BY ZEOLITES

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

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

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

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

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Ombrotrophic bogs are hydrologically isolated from the influence of local groundwaters and receive their nutrients exclusively from the atmosphere by dry and wet deposition. For that reason these systems have a great potential for recording the chronology of past atmospheric deposition. Unlike glacial archives, which reflect integrated long-range metals input, peat bogs can record dust supplied by local and regional sources, allowing the study of regional and local scale variability. Here we present the first data from Italian peat records reconstructed from a 13,221 yr cal BP peat core. The aim of our work is to reconstruct spatial and temporal variation of past climate and environmental conditions by a novel multi-proxy method characterized by high-resolution geochemical (trace elements, rare earth elements, Pb isotopes, organic carbon, humification) and pollen analysis using the Danta di Cadore 46°34'16'' N 12°29'58'' E and Coltrondo 46°39'28'' N 12°26'59'' E peat bogs from the north eastern Italian Alps. We propose a high resolution analytical approach based on the analysis of split sediments with the Avaattech XRF Core Scanner followed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis: we quantified major and trace elements in the peat profile, moreover we obtained lightness and colour parameters on the images recorded by a digital colour line scan camera connected to the XRF scanner. Conversion of element intensities measured by XRF core scanner to element concentrations is essential for quantitative applications involving mass-balance and flux calculations. Intensities of our XRF results were calibrated with control specimens taken from the same core at strata corresponding exactly to core-scanner measurements: total concentrations of elements were determined by using destructive technique employing microwave-assisted HF-HNO<sub>3</sub> digestion of peat. Samples treated in this way were subsequently analyzed by ICP-MS. Our determinations of specific element concentrations describe the trophic status of the peat profile and, together with a reliable age model combining <sup>14</sup>C dating with the independent <sup>210</sup>Pb dating, allow the reconstruction of rates and predominant sources of a variety of atmospheric trace elements. To our knowledge, this is the first attempt to provide quantitative geochemical interpretation of XRF core scanner data for this type of deposit. The results provide environmental information which is currently lacking in the north-eastern Italian Alps and more generally in northern Italy where a complete quantitative environmental record of major and trace elements from prehistory to the present has not yet been constructed.

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

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

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

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

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

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

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

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

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## SURFACE CHEMISTRY OF Ni-FREE STAINLESS STEEL

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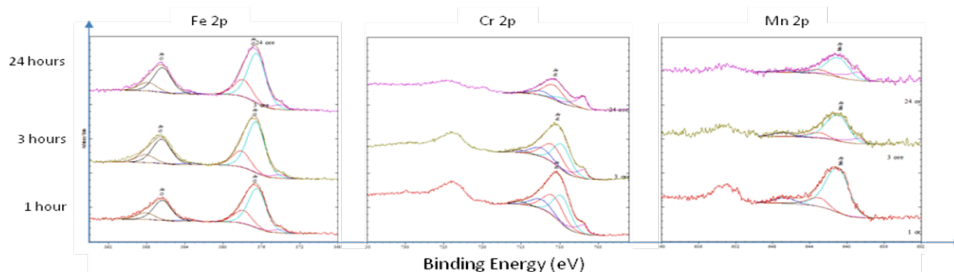
Nowadays there is a strong demand to replace CrNi stainless steels used for biomedical applications such as brackets in orthodonty with a new generation of more biocompatible austenitic, nickel-free alloys. The aim of this work is the assessment of the substances that leach from the steel and the investigation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

Time (h)	OCP ±20 (mV)		Rp (MΩ·cm <sup>2</sup> )	I <sub>corr</sub> (μA/cm <sup>2</sup> )
	t <sub>0</sub>	t		
1	-279	-257	0.334±0.003	0.156±0.002
3	-241	-221	1.185 ±0.007	0.044 ±0.001
24	-285	-151	2.92 ±0.06	0.02±0.01

mainly composed of oxides and hydroxides of Fe (III), Cr(III) and Mn (see figure). Fe (II) amount in the passive film decreases with immersion time in favor of Fe (III) oxide (Fe<sub>2</sub>O<sub>3</sub>) and

hydroxide (FeOOH). Cr as well is first present as Cr<sub>2</sub>O<sub>3</sub> and turns to Cr(OH)<sub>3</sub> for longer contact time to artificial saliva.

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



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

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

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

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

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

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

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

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

These examples show how the proposed bio-electronic platform, besides resulting in extremely performing biosensors, can open to gather insights into biological relevant phenomena involving interfacial modifications that can be electronically detected.

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## MULTIVARIATE STRATEGIES FOR SCREENING EVALUATION OF CHRONIC ALCOHOL ABUSE

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

## MULTIVARIATE CHEMICAL MAPS FROM $\mu$ -FTIR AND DESI-MS HYPERSPSPECTRAL DATA

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

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

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Aim of this work was to carry out an extensive characterization of the Oleoresin (OR) from *Copaifera langsdorffii* Desf. (Fabaceae), by thermogravimetric analysis (TGA), GC-MS profiling, and molecular mass determination. OR is used in the therapeutic management of several inflammatory affections, i.e. sore throat, urinary, gastric and pulmonary diseases, and to heal skin ulcers wounds. TGA was carried out under N<sub>2</sub> from 50 °C to 650 °C by a Perkin Elmer TGA7 Thermogravimetric Analyser. The DTGA thermal curve shows three peaks at 134 °C (22,7%), 248 °C (49,4%), and 371 °C (27,9%), each of them indicative of three main classes of components. Steam distillation of OR gives a volatile fraction (22% of the total), which when submitted to GC-MS analysis shows a set of sesquiterpene constituents, the main of which were  $\alpha$ -bergamotene,  $\alpha$ -himachalene,  $\beta$ -caryophyllene,  $\beta$ -elemene, cyclosativene,  $\beta$ -selinene, and paraffins. The bulk of these constituents, on the basis of their boiling points and total percentage content, very likely corresponds to the DTGA first peak. The residue was then submitted to derivatization (MeOH/6N HCl) and extracted with n-hexane. The extract, examined by GC-MS, exhibited a series of labdanic and labdenoic structures, diterpenoic acids, and diterpenes bearing  $\alpha$ - $\beta$  conjugated dienes, i.e. copalic, pimaric, isopimaric, abietic, daniellic, lambertinic, giberellic acids, the sum of them accounting to a 45% of the OR, close to the amount of the components present in the second TGA fraction. The average molecular weight (Mw) of the post n-hexane residue ( $\approx$ 35% of the total OR, coincident with the value of the last TGA peak), was determined on the OR sample with a multi-angle laser light scattering photometer (MALS, Dawn DSP-F from Wyatt) in off-line batch mode. The solvent used was ethanol at room temperature, the OR sample concentrations ranged from 0.7 mg/ml to 1.5 mg/ml. The MALS data analysis was performed using a conventional Zimm plot (i.e. a double extrapolation to zero angle and to zero concentration). The average molecular mass value was  $8835 \pm 660$  g/mol. The polysaccharidic nature of the polymer (a xiloglucanic structure<sup>1</sup>) was confirmed by the positive response to the Dubois reaction (phenol/H<sub>2</sub>SO<sub>4</sub>) specific for carbohydrates.

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

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

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

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

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

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Thirty-three Byzantine anonymous copper coins of century XI (“folles”) were investigated in order to determine their chemical composition both in the core and in the surface. The aim of this study was also the identification of correlations of coin composition in coins from different coinage periods. Inductively coupled plasma atomic emission spectroscopy (ICP-AES), a destructive technique, was employed for determination of major and minor metals (Cu, and Pb, As, Fe, Zn, Ag and Ni, respectively).

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

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

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

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

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

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

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

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

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

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

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

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

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## MERCURY ISOTOPE RATIOS AS CONTAMINATION MARKERS: PROCEDURE DEVELOPMENT AND APPLICATIONS

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

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## DETERMINATION OF WATER CONTENT IN ATMOSPHERIC PARTICULATE MATTER

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

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

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

The application of the method to real PM<sub>10</sub> samples has evidenced that the amount of water is subjected to very relevant variation, as a function of the PM chemical composition. Mass percentages of ca. 3-4 % have been obtained in most of the samples, but values up to about 15% have been reached when the chemical composition of PM was dominated by secondary inorganic ions and organic matter. Very different thermal profiles have been also evidenced. To try an identification of the type of the released water, the method has also been applied to some hygroscopic compounds (pure SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, NaCl, Na<sub>2</sub>CO<sub>3</sub>, ammonium salts and carbohydrates) that are likely present in PM.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

## AEROSOL CHARACTERIZATION BY PMF ANALYSIS OF SINGLE PARTICLE ATOFMS SPECTRA

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Reparto Carabinieri Investigazioni Scientifiche di Parma

The use of liquid chromatography coupled with mass spectrometry offers several opportunities for analysis in forensic chemistry.

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

Classical and new drugs like synthetic cannabinoids and cathinones, that constantly appear on the Italian market, counterfeit illicit medicines and Explosives residues have been analyzed to create and improve a ToF MS database. This allow the identification of different substances using “Targeted screening” based on retention time, on the exact mass (MS) and fragment product ions (MS<sup>E</sup>).

MS<sup>E</sup> is a novel, patented mode of data acquisition for Waters<sup>®</sup> Xevo G2ToF MS that provide a simple, unbiased, parallel route to delivering exact mass molecular (MS) and product ions in a single analysis.

Four new synthetic cannabinoids, used as ingredients for smart drugs, have been identified, during a survey. The characterization of these compounds has been made by gas chromatography-mass spectrometry (GC-MS), UPLC-HR-TOF, and nuclear magnetic resonance (NMR), leading to the identification of WIN48098, not yet found, to our knowledge, as adulterant in smart drugs, and AM679, AM2233 and JWH-307 identified in Italy for the first time.

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

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

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

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

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

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

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

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

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

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Luminescence detection is particularly attractive for bioanalytical applications and biosensors because it combines high detectability with simple instrumentation. Even if fluorescence, bio-chemiluminescence and electrogenerated chemiluminescence are the most common luminescence detection techniques, other techniques have been investigated over the years. Thermochemiluminescence (TCL), *i.e.*, the light emission originating from the thermolysis of a molecule, was proposed in the late ‘80s as a detection technique for immunoassays (1,2). However, after little pioneering work, TCL detection was abandoned due to methodological problems, such as the high temperature required to trigger the emission (200-250°C), and to the poorer detectability in comparison to other labels.

Herein, we report for the first time the TCL properties of an acridane-based 1,2-dioxetane showing a remarkably low (below 100°C) TCL triggering temperature and describe amine-functionalized TCL silica nanoparticles (SiNPs) incorporating this compound, either alone or together with a fluorescent energy acceptor, to be used as TCL labels in bioassays. Thanks to the signal amplification due to the high 1,2-dioxetane loading, the detectability of the doped SiNPs is comparable to that of enzyme labels. In addition, preliminary experiments showed that the doped SiNPs could be conjugated to antibodies maintaining their binding ability.

Therefore, these labels could pave the way for the revival of TCL detection in bioassays. Miniaturized, simple and ultrasensitive analytical devices and biosensors could be developed by taking advantage of the high signal/noise ratio of TCL detection and the possibility to perform the measurement without any addition of reagents.

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

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

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

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

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

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

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

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

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

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## METABOLOMICS OF TRANSGENIC PLANTS UNDER CHEMICAL STRESS

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

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

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

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

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

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

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

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## SURFACE ANALYTICAL CHARACTERIZATION OF MULTIFUNCTIONAL ZnO<sub>x</sub>-FLUOROPOLYMER NANO-COATINGS FOR THE TEXTILE INDUSTRY

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

In this study, X-ray Photoelectron Spectroscopy (XPS) has been used to quantitatively assess the materials' surface chemical composition, as a function of  $\phi$ . Six to seven carbon species could be detected in C1s spectra, the abundance of highly fluorinated moieties being inversely related to  $\phi$ . Two peaks were detected in the F1s spectra, and they could be attributed to inorganic, as well as organic species. Same was observed in the case of O1s XP regions. Zinc surface chemical speciation could be addressed by the indirect quantification of zinc fluoride and oxide species (by curve-fitting the O1s and F1s XP spectra), as well as by the direct study of the Zn L<sub>3</sub>M<sub>45</sub>M<sub>45</sub> Auger signals. As a result, the surface NP composition was interpreted in terms of the simultaneous presence of ZnO<sub>x</sub> and of small amounts of ZnF<sub>2</sub> species. Fourier transform infrared spectroscopy was used to characterize the materials' bulk composition, while transmission electron microscopy was used to morphologically characterize the nanocoatings. The antimicrobial properties of the ZnO-containing products were successfully demonstrated on two target microorganisms: gram-positive *Staphylococcus aureus* ATCC 25923 and gram-negative *Escherichia coli* ATCC 25922.

The promising results strongly encourage the application of ZnO<sub>x</sub>/fluoropolymer nanoantimicrobial coatings in the textile industry.

## GROWTH OF SULPHIDE THIN FILMS WITH TECHNOLOGICAL INTEREST

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In the last years research on renewable energy has become more important because of increasing of energy demand combined with the need of sustainability. In particular there is a big interest on photovoltaic energy and above all on thin film solar cell. Producing this kind of cell is cheaper compared to wafer-based solar cell. Current thin films solar cells are made of CdTe, a-Si or  $\text{CuIn}_x\text{Ga}_{1-x}\text{S}(\text{Se})_2$  (CIGS) but these materials are low abundant on Earth's crust and they could give toxicity problems. New materials like kuramite ( $\text{Cu}_3\text{SnS}_4$ ), kesterite ( $\text{Cu}_3\text{ZnSnS}_4$ ) and stannite ( $\text{Cu}_2\text{FeSnS}_4$ )-type materials are promising semiconductors for solar cells because of their suitable optical band gap, abundance and low environmental impact compared to CIGS CdTe and a-Si. Semiconductors are generally prepared by high temperature solidification methods from the elements in bulk form, or vapor phase and vacuum methods in the form of thin films[1][2]. The growth of these materials in bulk form or as thin films is also possible from liquid solutions. For this reason electrodeposition techniques are emerging especially as methods for the synthesis of semiconductor thin films and nanostructures[3]. Electrodeposition could be applied on large scale thanks to its economy, low environmental impact (we can work with aqueous solution) and the low working temperature. The electrochemical method of deposition ECALD (Electrochemical Atomic Layer Deposition) resulted a valid approach to prepare semiconductor compounds on metallic substrates. This technique is based on the alternate underpotential deposition (UPD) of atomic layers of the elements constituting a compound in a cycle that can be repeated many times to obtain the desired thickness. We realized thin films of ternary compounds  $\text{Cu}_x\text{Sn}_y\text{S}_z$  with different thickness and composition with ECALD technique. The obtained films were characterized by AFM, XPS, stripping voltammetry and UV-vis spectroscopy. AFM measurements showed a similar morphology of the film compared to the substrate. From XPS analysis we observed that the stoichiometric Cu/Sn ratio changes if we perform different numbers of cycles of each metal, even if the ratio is whenever shifted to Cu. The band gap values of the film estimated with UV-vis experiments are suitable as semiconductors in solar cells.

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

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

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

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

Cyclic voltammetry, chronoamperometry, surface plasmon resonance and atomic force microscopy were used to characterize the modification of surface and to investigate the bioelectrocatalytic response of the biosensor. A tentative application of the developed enzymatic electrode was performed evaluating the detection of some phenolic compounds by using enzymes above mentioned (3).

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

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## ADVANCED DETECTION OF GENETIC DISORDERS BY SURFACE PLASMON RESONANCE IMAGING

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

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

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

The new catalyst became fully characterized by a combination of FTIR, NMR, and XPS spectroscopic techniques. In particular, XPS has been used for the surface characterization of both the ketone catalyst as well as its precursor, i.e. the silica-CO<sub>2</sub>H. Besides the expected F1s signal (688.3 eV) in the wide scan spectrum of the catalyst, the high-resolution C1s region presents new contributions at 286.6, 288.4, and 292.7 eV. Consistent with literature (4), the latter two components are assigned respectively to C(:O)CF<sub>3</sub> and to CF<sub>3</sub> groups. Useful hints concerning the fate of catalyst after exhaustive working could be gathered by quantitative <sup>19</sup>F NMR and XPS analysis. For instance, on going from the initial active catalyst to its almost exhausted form, the XPS C1s spectrum showed a significant drop of the carbonyl (288.4 eV) and CF<sub>3</sub> (292.7 eV) contributions.

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

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

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

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

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

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

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

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

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

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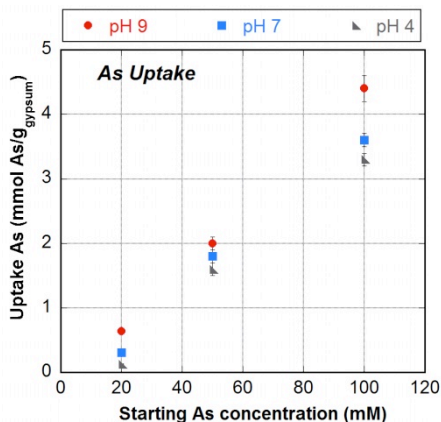
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## ARSENIC REMOVAL BY INTERACTION WITH GYPSUM: EFFECT OF PH, AS (V) CONCENTRATION AND PARTICLE SIZE.

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Arsenic-bearing sulfide minerals give rise to great environmental concern owing to the risk of toxic As release as effect of their oxidation/dissolution. [1]. According to the European regulations (e.g. Council Directive 98/83/EC) As content in drinking water has to be lower than  $10 \mu\text{g}/\text{dm}^3$ . Arsenic can be removed from water by several methods [2] and in this work the immobilization of arsenate ion on gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) was investigated. Gypsum was chosen for two main reasons: 1) its low cost, 2) it is often present in mine wastes together with arsenic minerals thus it might contribute at arsenic immobilization together with ferrihydrite [3]. Arsenic removal by gypsum was investigated by means of ICP-OES and AAS as a function of: arsenate concentration (20mM, 50 mM, 100mM), pH (4, 7, 9) and gypsum particle size (ranging between  $40\mu\text{m}$  and  $4800 \mu\text{m}$ ). The removal was found to follow a pseudo-second order kinetic. Best results were obtained at pH = 9, starting with higher arsenic concentration and with



gypsum particle size between  $800 \mu\text{m}$  and  $2000 \mu\text{m}$ . Due to the solubility of precipitated calcium arsenates the concentration of arsenic in the solutions was always higher than  $10 \mu\text{g}/\text{dm}^3$ . Arsenic removal by gypsum might be used as a first, cheap, method to decrease arsenic content in highly contaminated water.

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## APTAMERS IN BIOSENSORS: RECENT ADVANCES AND POSSIBLE APPLICATIONS

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

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

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

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

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

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

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

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

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

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

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

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

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

The use of TripleTOF<sup>TM</sup> high resolution mass spectrometer helps in the elucidation of the unknown chemical structures also by using powerful software to mine the recorded chromatogram.

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

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

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

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

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## Posters

## **A NEW ANALYTICAL METHOD FOR THE DETERMINATION OF POLYPHENOLS BY LIQUID CHROMATOGRAPHY AND PULSED AMPEROMETRIC DETECTION AT GLASSY CARBON ELECTRODES**

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Polyphenols are a group of secondary metabolites, widely distributed in the plant kingdom, whose quantitative determination plays an important role in food science because of their anti-carcinogenic, anti-thrombotic and anti-inflammatory properties. Current analytical methods mainly include liquid chromatography coupled with UV-detection and mass spectrometry that require extensive sample clean-up steps (1,2). Also, methods based on electrochemical detection at constant potential with glassy carbon, and pulsed amperometric detection (PAD) at gold or platinum electrodes represent a valid alternative in terms of instrumental costs and simplicity of operation, but they suffer from electrode fouling problems (3) or show high signal to noise ratios (4). In this study, a new method based on reverse phase liquid chromatography and PAD at a glassy carbon electrode (PAD<sub>GCE</sub>) was developed for the simultaneous determination of flavonoids and phenolic acids in real samples. Chromatographic experimental conditions, such as column features, mobile phase composition, gradient elution and flow rate were carefully optimized. The separation was performed by using a core-shell C18 column, eluted in a linear-concave binary gradient. The selection of the potential-time parameters was carried out by hydrodynamic voltammetry, based on multiple injections of the mixed standard solution of polyphenolic compounds. The optimized triple-step potential waveform provided an efficient detection, with a good long-term reproducibility that confirms the elimination of the electrode fouling. The method, which allows the efficient separation of polyphenols in less than 25 min, was extensively validated, and the analytical performances of linearity, selectivity, precision, and detection limits (0.003-0.4 mg/L) demonstrates the method feasibility in accurate confirmation analyses. The potential of the proposed RPLC-PAD<sub>GCE</sub> method was assessed by the determination of polyphenols in artichoke bracts extracts and olive mill wastewaters, with a minimal sample preparation.

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## 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? – PART II.

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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. The knowledge of a chemistry relationship between the soil and the agricultural products is an important tool for the quality assessment of food. Metal cations onto particle surface of soil changing the environmental conditions can be mobilized and therefore to become bioavailable.

In particular the chemical behavior of YLOID (Y, La and Lanthanoid) was studied to evaluate and trace the distribution from soil to roots, leaves to the grape in *Vitis vinifera* L. In a first study YLOID, present in equimolar amount in the growth substrate, suggested no preferential sorption of any element in overall root samples and similar pattern was also found in epigeal samples (1). Therefore the grapevine could be a potential proxy of different YLOID distributions in different soils. For these reasons are in progress experimental trials to verify if different varieties of rootstock on identical soil have similar behavior and if the same rootstock could reproduce different YLOID distribution on different soil typologies. Three different varieties of rootstocks *V. berlandieri* X *V. rupestris* (1103 Paulsen, 779 Paulsen and 140 Ruggeri), planted on different soils (carbonatic, clayey and volcanic soils), are under observations. The uptake of YLOID and their distribution in grapevine system were studied under controlled conditions following the plants growth in pots. The experimental system consisted of a set of 81 vines (27 per rootstocks) implanted, in groups of nine, on the three different types of soil.

Three replicates for each group and for three different phenological stages were sampled. To study the metal distribution, the main part of plants: roots, stem, shoots and leaves were analysed. The obtained results were critically discussed on the basis of the different amount presents in all parts of plants.

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## **ROOM TEMPERATURE IONIC LIQUIDS AS PROFITABLE OVERLAYERS FOR THE QUARTZ CRYSTAL MICROBALANCE ESTIMATION OF FOOD QUALITY BY THEIR ODOR ANALYSIS**

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Odor analysis has received a growing interest in recent years owing to its large concern in environmental protection, public health, food process monitoring and food quality control. Analysis of the whole odor, can provide in particular valuable information on food quality and safety, which are especially welcome when achieved by simple, rapid, on-line and real-time detection methods. With this aim most electronic noses have been proposed. These devices provide responses by combining output from arrays of non-specific chemical sensors to produce a fingerprint of assayed samples which is statistically analysed often using multivariate analysis and neural network techniques. They are frequently assembled by sets of metal oxide (MOSFET), surface acoustic wave (SAW) and quartz crystal microbalance (QCM) gas sensors.

We propose here an array of QCM sensors filmed with different RTILs overlayers for the analysis of flavours with a complex composition. Seven RTILs, all containing imidazolium or phosphonium type cations but differing from one another in the length and branching of alkyl groups and in the anion, were adopted for the detection of more than 30 VOCs. Responses provided by this array produced proper fingerprints which were statistically processed by the simple principal component approach (PCA) which allowed different classes of VOCs to be discriminated. This QCM array was applied to six different real cinnamon samples, proving that its use allowed these samples to be distinguished on the basis of their botanical classification

## **MEASUREMENTS OF ANTIOXIDANT CAPACITY OF FRESH BLUEBERRY AND BLUEBERRY BASED INTEGRATORS, USING BIOSENSOR, SPECTROPHOTOMETRIC AND FLUORIMETRIC METHODS.**

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The antioxidant compounds contained in many foods and beverages are capable of reacting with the radicals and thus play an important role in the prevention and defence against oxidative diseases, representing a protective factor of fundamental importance for human health. Sometimes, in the case of incorrect food habits or physical deficiencies, food intake alone is not sufficient to provide enough antioxidant nutrients. Therefore, in such cases, the use of food integrators is recommended. This has become a widespread practice although the antioxidant properties of these compounds are often not fully quantified. It is thus of particular and topical interest to be able to come up with new analytical methods to assess the antioxidant capacity of the various 'over the counter' products available in drugstores and that may be purchased without medical prescription. The aim of the present work was to investigate the antioxidant capacity of capsules containing blueberry based products which are included among the group of integrators most widely sold in drugstores owing to this capacity and produced by various drug firms. The results of the investigation are compared to rank these products in the order of their antioxidant capacity. Black blueberry leaves and berries are rich in active principle, they contain antioxidant compounds like tannins, flavonoids, anthocyanins, glucosides and anthocyanidins. It is therefore its antiradical action that is of fundamental importance: in order to measure antioxidant capacity, in addition to the various methods described in the literature, our laboratory has recently developed a special electrochemical method based on a superoxide dismutase (SOD) biosensor (1) to determine the superoxide radical. The results obtained by applying the SOD biosensor method to various blueberry based integrators were compared with the results obtained with the spectrophotometric method based on N,N-dimethyl-p-phenylenediamine (DMPD- FeCl<sub>3</sub>). The same results are currently being compared with those obtained also using the ORAC fluorimetric method, the most frequently used to determine antioxidant activity in food matrices and adopted as reference method. It is also planned to compare the antioxidant capacity of these integrators sold in drugstores with that of fresh blueberries, with and without skin and seeds.

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## **LACTOFERRIN DETERMINATION USING IMMUNOSENSOR METHOD BASED ON SURFACE PLASMON RESONANCE (AND TWO DIFFERENT TYPES OF MEASUREMENTS). COMPARISON WITH PREVIOUS IMMUNOLOGICAL METHODS**

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In mammalian secretions such as milk, tears, saliva and seminal fluids Lactoferrin is an important iron-binding glycoprotein, present in large quantities; it is also contained in powdered milks for babies sold in drugstores. Important biological functions have been attributed to this protein, including antibacterial and anti-inflammatory activity. Several immunosensors for lactoferrin determination were fabricated by us in previous researches [1] using different construction techniques and measurement patterns. At present, our team is testing the feasibility of constructing a new immunosensor for lactoferrin analysis based on surface plasmon resonance (SPR) in the Kretschmann configuration, but using two different devices, operating in batch, or in flow mode. In previous researches [1] “competition” immunological procedures were used in most cases; conversely, the SPR transduction technique used in the present research allowed a “direct” measurement procedure to be used, whatever batch or flow mode was used for the measurements.

If operating in batch the SPR experiments were performed using an ESPRIT instrument (Echo Chemie B.V., The Netherlands). The antibody-modified surface yielded a first calibration curve for the lactoferrin with a linearity range of 0.1 to 2.0 mM and about 0.07 mM LOD. When on the contrary measurements were performed using the flow operating mode, a Biosuplar 400T (Analytical  $\mu$ -Systems - Dep. of Mivitec GmbH, Sinzing, Germany), was used. In this case a calibration curve for Lactoferrin showed a linearity range of 0.1 to 10  $\mu$ M and about 0.05  $\mu$ M LOD.

The measurement time, using both two measurement mode, was found to be about half that required in previous competition methods. A detailed comparison was made of the analytical features of new devices with those ones of previously developed immunosensors and the advantages and disadvantages of the new SPR methods investigated.

Lastly several applications and comparison were carried out on cow, goat milk and on different type of dried milk for babies.

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## **DETERMINATION OF PROLINE IN HONEY: COMPARISON BETWEEN OFFICIAL METHODS AND OPTIMIZATION OF THE ANALYTICAL METHODOLOGY**

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The analytical methods used in the literature for the determination of proline, the predominant free amino acid of honey, refer to the official methods of the International Honey Commission (1) and of the Association of Official Analytical Chemists (2). These methods are derived from the original method of Ough (3), in which the content of proline was measured by spectrophotometry from the colour developed with ninhydrin at a wavelength of 510 nm. While the AOAC method follows the original procedure strictly, the IHC method introduces some changes, the most important of which refers to a water bath at 70 °C for 10 min following the boiling bath included in the original method. The comparison of results obtained by the IHC method with those obtained by the original method does not show statistically significant differences, thus the extra treatment stages in the IHC method, which can lengthen the time of analysis, are pointless. We also demonstrate that background interferences due to honey matrix are significant, thus a subtraction procedure of the sample absorbance should be used, as provided for in the AOAC method, using a solution containing the sample and all the reagents without ninhydrin. The method was optimized for the waiting time from the addition of the last reagent and the absorbance recording, which is found to be 35 min. The limit of detection in honey solutions is ~20 mg/L, about 2-fold higher than in aqueous solution, and the calibration curves obtained in the two matrices have the same slope. As regards accuracy, good results are obtained for recovery in honey solutions (~105% and ~95% for low and high quantity of added proline), and the same is true for the precision (repeatability ~2%, reproducibility ~4%). The method was applied to 43 unifloral honey samples from the Marche region, Central Italy. Proline content was consistent with reported mean values found in the literature for the respective typologies of unifloral honey.

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## **ISOTOPIC RATIO MASS SPECTROMETRY (IRMS) FOR CHARACTERIZING ORGANIC OLIVES.**

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Foods recognized as "organic" must be grown and processed according to local regulations (EEC) 2092/91(1). This regulation describes the (i) agricultural practices allowed in organic crops and (ii) the control system that must be implemented to ensure that these conditions are met. Allowed fertilizers include animal manures, compost and other products of both animal and vegetable origin. Recent studies have shown that the presence of synthetic fertilizers, used in traditional agricultural regimes, could be detected by the evaluation of the isotopic composition of nitrogen (2-3). In light of these results, it was decided to develop a method for the evaluation of the isotopic ratio of nitrogen in olive pulps. Isotopic parameters ( $\delta^{15}\text{N}/^{14}\text{N}$ ) were investigated as potential markers of organically cultivated olives produced in Calabria region in the 2011 crop year in certified farms. The cultivar considered were: Nocellara, Tondina, Dolce di Rossano, Cassanese, Carolea and Roggianella. The isotopic ratio of nitrogen is shown to be a significant variable for distinguishing between organically and conventionally cultivated olives.

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## **MODIFIED ELECTRODES AND MICROELECTRODES FOR THE ANALYSIS OF FOOD MATRICES. DEVELOPMENT OF AN ELECTRONIC TONGUE**

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In recent years growing attention has been focused on the development of analytical instruments meeting the requirement of simple instrumentation, rapid response, low cost, and even portability. Electrochemistry can give important contributions to this topic, offering both specific sensors for the direct determination of analytes of interest, and sensor arrays for blind analysis, in the frame of the so-called electronic tongues (ETs). In this context, we are focusing the attention on the development of poly(3,4-ethylenedioxythiophene) (PEDOT) polymers and PEDOT based composite electrodes to use in both analytical approaches. In particular, as to blind analysis, we have recently demonstrated the advantages offered by the use of PEDOT sensors in ETs for the recognition of different kinds of fruit juices [1], and for the discrimination of white and red wines with respect to variety, geographical origin and even to some physico-chemical parameters of interest in the examined samples [2-4].

On the basis of our previous results, we are now extending the possible applications of the developed electrochemical system to a wider range of food samples, i.e. even to dense or low conductive matrices such as edible oils and dairy products, namely yogurt. In these challenging tasks, the use of PEDOT modified microelectrodes reveals to be particularly profitable. In view of approaching these applications with best efficiency, the necessity to perform an exhaustive study of the sensing system is urgent. The goal is to define the optimal experimental conditions for the electropolymerisation process, in order to enhance the electroanalytical performances of these particular devices in complex media such food samples.

## **ELECTROCHEMICAL EVALUATION OF EXTRAVERGIN OLIVE OIL'S TOCOPHEROL AND POLYPHENOLS IN ORGANIC PHASE**

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Virgin olive oil is a valuable source of natural antioxidants. It presents resistance to oxidative deterioration of fatty acids, is characterized by a high monounsaturated to polyunsaturated ratio, and contains a large amount of compounds with powerful antioxidant activity, including  $\alpha$ -tocopherol and polyphenols. Polyphenols are highly variable in terms of structure and concentration, depending on the olive cultivar, on the extraction technology, and on the olive oil storage period and condition. The polyphenolic pattern may provide an indication of the olive oil quality as a whole. In this communication we present preliminary results on the evaluation of different electrode materials (bare glassy carbon and differently modified glassy carbon) as working electrodes for the electrochemical detection (by differential pulse voltammetry) of  $\alpha$ -tocopherol and polyphenols in mixed organic solvent medium. Using glassy carbon as the working electrode, linear sweep voltammetric curves show peaks due to oxidation of  $\alpha$ -tocopherol, o-diphenolic molecules, and monophenolic molecules (at 250, 430 and 630 mV vs. Ag/AgCl, respectively); partial overlap between the signals due to o-diphenol and  $\alpha$ -tocopherol oxidation is observed in the 250 mV region. Further work is needed to use the proposed electrochemical approach for the realization of a PLS regression model for the quantitative analysis of  $\alpha$ -tocopherol, o-diphenols and monophenols in olive oil samples. In this context the use of a set of bare and differently modified glassy carbon electrodes can be particularly profitable, each electrode providing, in principle, different information on the solution under analysis. On the basis of our previous results (2) and of the study in progress in our laboratories, the choice of poly(3,4-ethylenedioxythiophene) and graphene as electrode modifiers has been made.

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## EVALUATION OF THE ANALYTICAL PERFORMANCES OF A PEPTIDE BASED ELECTRONIC NOSE TOWARDS SOME TYPICAL AROMA MOLECULES AND FOOD SAMPLES

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The complexity of the aromatic patterns released by foods is mainly dependent on the original volatile molecules composition and product matrix. Modification of volatile compounds occurring during the production process should be monitored to ensure the quality of the final product.

Electronic nose has proved to be a very useful tool for food and aromas analysis (1). In this work quartz crystal microbalance (QCM) based array of gas sensors has been modified using a novel approach. The QCM surfaces have been covered with Gold Nano-Particles (GNPs) bearing short peptide moieties chosen for their ability as mimicking agent of real biological receptors. The array was composed of seven modified QCM: one carrying only GNPs without the peptide moiety, and six modified as follows: GNP-Cys-Gly, GNP-Glutathione, GNP-Cys, GNP- $\gamma$ -Glu-Cys, GNP-thioglycolic acid and GNP-Cys-Glu-His-Gly-Gly-Pro-Ser. The GNPs were synthesized using the NaBH<sub>4</sub> method and characterized using TEM and VIS spectroscopy. Response of the sensors array to the samples has been characterized by head-space analysis and nitrogen as carrier.

Frequency shift data have shown the ability of the electronic nose to discriminate different solvent, and different aromas in model solution of the same solvent, just after a simple principal component analysis.

Real extra-virgin olive oil samples have also been tested and data has been compared with GC analysis data and panel tests. In this case, the peptide-based electronic nose, easily discriminated defected samples. Data on other food samples such as candies will be also presented using a slightly modified GNP-peptide array of sensors, including three new developed penta-peptides (GNP-Cys-Arg-Gln-Val-Phe, GNP-Cys-Ile-His-Asn-Pro, GNP-Cys-Ile-Gln-Pro-Val).

Considering the ease of synthesis and the wide range of peptides with different binding ability, this approach appears very promising for the development of a new generation of e-noses, based on different sets of peptides.

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## INFLUENCE OF CULTURAL PRACTICE AND POSTHARVEST DRYING PROCESS ON OCCURRENCE AND CONTENT OF PHENOLIC COMPOUNDS IN GRAPE BERRIES

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The phenolic compounds constitute one of the most numerous and widely distributed groups of substances in the plant kingdom. These compounds are present in grape and wine with an essential role in enology. The phenolic composition of grapes are directly related to the quality of wine and contribute to its organoleptic characteristics such as color, taste, astringency and bitterness. In addition, the content of phenolic compounds may comprise a source of molecular markers for studying the metabolic processes taking place both during the fruit ripening that the postharvest dehydration processes to which are exposed the grape for the production of dessert wines (1). This communication discusses the results of a study undertaken to evaluate the change in occurrence and content of phenolic grape berries in response to changes in the physiological activity of the plant as a result of variations in the microclimate heat and light, due to different exposure to solar radiation of the bunch. Changes in the microclimate was carried out with defoliation of the vine made in subsequent moments of development of the berries. The data presented were produced to evaluate the effect of defoliation performed at the stage of fruit set and maturity on the accumulation of phenolic compounds in grape. The thesis of defoliation performed on Nebbiolo grapes (*Vitis vinifera L*) have been compared with a control consisting of grape berries harvested from no defoliated plants. Subsequently, these thesis were subjected to dehydration process at three different temperatures; 10, 20, or 30°C in order to evaluate the possibility to stimulate the biosynthesis of polyphenol compounds by applying different rates of postharvest water loss. The simultaneous identification and quantification of the major phenolic compounds occurring in the whole berries of grape was performed by high performance liquid chromatography using a narrow bore reversed phase column (2.0 mm I.D.) and detection by in sequence UV-visible photodiode array spectrophotometry, equipped with a semimicro detection cell, and electrospray ionization mass spectrometry. The ESI-MS detection was performed in negative ionization mode for phenolic acids, flavonols, catechins and stilbenes and in positive mode for anthocyanins.

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## DETERMINATION OF PHENOLIC COMPOUNDS IN COMPLEX MATRICES

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Determination of phenolic compounds in complex matrices was performed using a molecularly imprinted polymer (MIP) as sorbent material in a solid phase extraction (MISPE). MIPs are functional polymers generated by molecular imprinting, an efficient method for producing materials with specific recognition sites. The technique consists of self-assembly of a functional monomer and a template molecule in solution followed by co-polymerization of the functional monomer with an excess of an appropriate cross-linking monomer. After removal of the template, the resulting polymer exhibits high affinity for the molecule used as template and structural analogues. In the recent years MISPE has been successfully applied to solve several challenging issue in food, biological and environmental analysis. Components of food matrices and beverage have been of considerable interest in recent years because of their potential utility as pharmaceutical agents for their antioxidant and anticarcinogenic activity (1,2).

Synthesis of a selective MIP was achieved using (E)-resveratrol as the template, 4-vinyl pyridine (4-VP) as the monomer, ethyleneglycol dimethacrylate (EGDMA) as the crosslinker and 2,2'-azobisisobutyronitrile (AIBN) as the inizerator (3).

The solid phase extraction protocol consists of three steps: sample loading, washing by mean of acetonitrile and elution with methanol. The phenolic compounds such as quercetin, apigenin, luteolin and caffeic acid were selectively extracted from these matrices because of their structural similarity to (E)-resveratrol. Separation of phenolic compounds was performed by reversed phase high-performance liquid chromatography (RP-HPLC) coupled to UV and MS detection.

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## **DEVELOPMENT AND VALIDATION OF HPLC METHODS FOR THE DETERMINATION OF FOOD ANTIOXIDANTS NOT ADMITTED IN FRESH MEAT PREPARATIONS**

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Fresh meat preparations rapidly oxidize in the presence of oxygen reducing the shelf-life and compromising the product attractiveness. In order to remedy this inconvenience the addition of some chemical additives with antioxidant activity was often used in the past (1, 2, 3). These additives are: ascorbic acid and its salts (ascorbates), sulphiting agents and nicotinic acid. The addition of these additives is a food sophistication because it is not allowed by the actual legislation. Only ascorbic acid and ascorbates may be added in fresh meat preparations but only in prepacked products (4). Laboratories in charge of food products official controls have to verify the absence of not allowed food additives and they have the necessity to use reliable analytical techniques for these determinations. In this work three analytical methods have been optimized and validated for the determinations of ascorbic acid, sulphiting agents and nicotinic acid in fresh meat preparations. The reversed phase liquid chromatography with U.V. Diode Array Detection was used for the determinations of ascorbic acid and nicotinic acid, while ion chromatography with conductometric detection was used for the determination of sulphiting agents. The methods have shown the requested specificity for meat products analysis, a good accuracy (mean recoveries in the range 82.2-114.0% and CVs% lower than 6.0%) and low measurement uncertainties. Moreover they are very simple and rapid with a maximum execution time of 60 minutes.

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## STUDY OF GRAPE METABOLOMICS BY “SUSPECTS SCREENING” ANALYSIS

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“Suspects screening” is a targeted metabolomics approach in which the identification of metabolites relies on available compound-specific information such as molecular formula and structure (1). This method has been applied to the study of metabolomics of different grape varieties. Grape berries were extracted with methanol and liquid nitrogen and the extract was analyzed by accurate mass measurement using a 40.000-resolution Liquid Chromatography-Quadrupole Time of Flight Mass Spectrometry (LC/QToF) system.

Metabolites were identified by the library *GrapeMetabolomics* constructed by including the information of the literature and the data of potential grape metabolites found in the available electronic databases. Partial confirmation of the library hits was achieved by identification of metabolites in some grape varieties previously studied taken as models for their peculiar chemical characteristics (Raboso Piave for polyphenols, Moscato Bianco for aroma precursors) (2). Currently *GrapeMetabolomics* contains around 1000 putative grape compounds with molecular weight between 100-1700. When untarget analysis suggests a new compound, it is added to the library, as a consequence a further expansion of *GrapeMetabolomics* is probable. Compounds are identified on the basis of accurate mass and isotope pattern, next confirmation is provided by multiple mass spectrometry (MS/MS).

Depending on the grape variety between 260-390 signals were assigned to putative compounds, mainly including nutraceutical and antioxidant compounds such as anthocyanins, flavones and flavanones, procyanidins, phytoalexins, phenolic acids. Sixteen stilbenes and derivatives were identified (resveratrol, piceid, several viniferins, resveratrol dimers, trimers and tetramers) together with large number of aroma precursors, primary metabolites and peptides. In general, between 30-60 hits had identification score higher 99%, and more than 100 hits higher 95%.

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## **RADIOSTRONTIUM ANALYSIS IN CHEESE SAMPLES: DEVELOPMENT AND VALIDATION OF A RADIOCHEMICAL METHOD BY LIQUID SCINTILLATION COUNTING (LSC)**

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$^{90}\text{Sr}$  is one of the most hazardous pollutants produced in nuclear fission processes since its long physical and biological half-life (28.8 years). Radiostrontium decays to its radioactive daughter nuclide  $^{90}\text{Y}$  emitting high-energy beta particles. When introduced into the environment  $^{90}\text{Sr}$  migrated through food-chain into the milk and dairy products and the accidental ingestion of contaminated food leads to a rapid absorption of the radioisotope that deposit in bones posing a high risk to human health. To detect low activity levels of  $^{90}\text{Sr}$  in cheese samples a radiochemical procedure using ultra low level liquid scintillation counting (LSC) was developed and validated. The most difficult analytical step is the separation of  $^{90}\text{Sr}$  from other alkaline earth elements, particularly calcium. The yttrium separation is easier than strontium since it can be selectively extracted with Bis(2-ethylhexyl)phosphate and then precipitated as oxalate until achievement of  $^{90}\text{Sr}/^{90}\text{Y}$  secular equilibrium. Moreover, LSC provides information about the energy distribution of the  $^{90}\text{Y}$   $\beta$ -spectra with high counting efficiency (89%) and very low background. Trueness, precision, selectivity, ruggedness and measurement uncertainty were evaluated following an in-house validation model. The validation parameters were determined using hard cheese samples fortified with known activities of  $^{90}\text{Sr}$  (1 Bq/kg). The repeatability values were calculated in terms of CV%, corresponding to 11%, with an average recovery of 96%. Decision Threshold and Detection Limit were also calculated in compliance with ISO 11929:2010 and correspond to 0.003 Bq/kg and 0.008 Bq/kg respectively ( $\alpha = \beta = 0.05$ ). Ruggedness of the method was demonstrated analysing soft and semi-soft cheeses of different type spiked at a level of 1 and 0.5 Bq/kg. In order to assess the reliability of the analytical procedure for routine analyses of  $^{90}\text{Sr}$ , 14 samples of ripened cheeses, obtained from goat and sheep milk, were analysed. The results of validation process show that LSC method is efficient and reliable at very low levels instead of conventional procedures that use gas proportional counter (1).

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## TRACING THE ORIGIN OF MILK AND MILK PRODUCTS: A SIMPLIFIED PROCEDURE FOR EXTRACTION/ISOLATION OF GLYCEROL FROM WHOLE MILK AND GC-IRMS ANALYSIS

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Recently the consumer demand of transparency and attention on foodstuff quality and safety is increasing, and, related to that, the need of qualified origin products on the market (1). Livestock feeding regimen, breeding conditions and growing environment are parameters that need to be traced to reveal origin, not only geographic, in order to certify quality, authenticity, and typicality of dairy products. Isotope ratio analysis was demonstrated to be a suitable tool for determining the origin of food, milk and dairy products included (2). It is known that the ratio  $^{13}\text{C}/^{12}\text{C}$  of animal products is correlated to animal diet since it discriminates between  $\text{C}_3$  and  $\text{C}_4$  plants (3), and in particular  $^{13}\delta$  of glycerol has been shown to increase with maize amount (4). The possibility to trace OGM presence in the fodder can be speculated on the same hypothesis, since transgenic fodder is mainly made up of soybean, a typical  $\text{C}_3$  plant. Milk samples from pasture-fed and silage-fed cows were collected from the Italian market and analyzed for  $^{13}\text{C}/^{12}\text{C}$  ratio of glycerol. A sample preparation from whole milk was set up, specifically targeted for GC-IRMS analysis. First, proteins were precipitated, then fat was separated, and through saponification glycerol was released from triglycerides and then isolated from fat components such as fat acids. After derivatization the acetylated mixture was purified in HPLC-UV. The purified fraction collected underwent GC-IRMS analysis. When compared to milk from silage-fed cows, milk from pasture-fed cows resulted enriched in  $^{13}\text{C}$ , showing a different diet regimen, rich in  $\text{C}_4$  plants, and therefore suggesting a GMO-free diet. Future developments of the present work will include the investigation of different isotope ratios and other parameters such as fatty acids compositional profile and screening of milk for exogenous DNA presence. Finally the whole data collected will be processed with chemometric methods.

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## TARGETED PROTEOMIC APPROACH FOR TRACE MULTI ALLERGEN DETECTION IN FOODS

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Mass spectrometry (MS)-based methods play a pivotal role in proteomic research, being applied as confirmatory tools for unambiguous identification and characterization of proteins and peptides thanks to their high specificity, sensitivity and accuracy. The presence of allergens could be intentional or could occur accidentally *via* cross-contamination at any stage of food production, so that legislation in several countries, especially in the USA and EU, was put in place to increase food safety (1). In this context, enhanced sensitivity for the simultaneous determination of five nut allergens in biscuits and in the dark chocolate complex matrix was obtained by introduction of a rapid size-exclusion solid phase extraction-based step before liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS<sup>2</sup>) analysis. Allergenic proteins Ana o 2 (cashewnut), Cor a 9 (hazelnut), Pru 1 (almond), Jug r 4 (walnut) and Ara h3/4 (peanut) were characterized after enzymatic digestion of the extracts by MALDI-TOF-MS and LC-MS<sup>2</sup> for the selection of specific and unique targeted peptides. A very fast and efficient separation (<12 min) of marker peptides with selected reaction monitoring (SRM) detection was obtained. Limits of detection in the 0.1-1.3 mg nut/kg and 5-15 mg nut/kg ranges for biscuit and dark chocolate samples as well as high recoveries (84(±6)-106(±4)% for biscuits and 98(±5)-108(±6)% for dark chocolate) proved the excellent capabilities of the exploited sample treatment method combined with the LC-MS<sup>2</sup> analysis. Good precision in terms of intra- and inter-day repeatability was calculated, being always lower than 19% (*n*=75). Linearity was demonstrated up to four and three orders of magnitude for biscuits and dark chocolate, respectively.

Finally, the validated method was successfully applied for the identification and absolute quantification of hidden nut traces in ten samples of different brands of biscuits and chocolate aiming to ascertain possible discrepancies between allergen content and food allergen labelling. The five biscuit samples were negative, whereas two of the five dark chocolate samples under investigation resulted positive to hazelnut, thus remarking the need of reliable analytical methods for strict multi allergens quality controls.

Compared to commonly used single-allergen immunological assays, this rapid and sensitive method opens the way of LC-MS/MS SRM multi allergen analysis to food safety testing programs.

(1) European Commission, Off. J. Eur. Union L368 (2006) 110

## EVALUATION OF SAFETY AND QUALITY OF FOOD BY CZE-MS

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The coupling of capillary electrophoresis to mass spectrometry (CE-MS) has enhanced the potentiality of this separative technique giving a new challenge in many fields of analytical chemistry, as demonstrated by many recent papers.

In this presentation coupling of a CZE system to mass spectrometry (MS) via an electrospray ionization interface (ESI) for the determination of selected markers of interest in the field of food safety, as well as for monitoring food technological processing and its quality has been proposed.

In detail, we focused our study on the following topics:

Furosine and hydroxymethylfurfural (HMF) A known are markers of food processing, largely employed to control thermal treatment and evaluate product quality. HMF is also under investigation for toxicological concern since it was shown to have cytotoxic, genotoxic, and tumoral effects. We optimized and validated a new method for qualitative and quantitative analysis of furosine and HMF in food products by CE coupled to MS-MS. Despite all previous CE methods proposed in literature for furosine analysis, no SPE treatment was required. The method has been applied to the analysis of different food products such pasta, milk, *tigelle* bread and flour, with particular attention to infant food.

Melamine. We studied the dependence of melamine and cyanuric acid mobility on several parameters, and developed a rapid method for their evaluation in food products. The procedure is also suitable for the control of melamine traces released by cutlery, pointed out by the EFSA alert in 2012.

Lysozyme and natamycin. They are currently used in food technology as additive for their antibacterial activity. Lysozyme is widely employed in winemaking process to control the growth of lactic bacteria. Natamycin is a natural antifungal agent produced during fermentation. Both can be used to incorporate antimicrobials agent to prepare active packagings.

For all the proposed applications the analytical conditions, including capillary length, background electrolyte concentration and pH, applied voltage, sheath liquid, have been optimized, and the proposed and validated methods have been applied to the quantitative determination of the selected markers in different food products as well as to study the development of innovative active food packaging films.

Our developed and validated methods have been proposed as powerful analytical tools regarding the and valid alternative to LC-MS.

In order to test the reliability of our methods, a comparison of some results with data obtained by LC has been carried out. Advantages of CE regards mainly economic impact since few amount of solvent is required, and cheap capillaries are employed instead of dedicated columns whose life is limited.

**Keywords:** Capillary electrophoresis-mass spectrometry; Melamine; Furosine; HMF; Lysozyme.

## **DEVELOPMENT OF AN ANALYTICAL METHOD LC/PAD FOR THE ANALYSIS OF SULFONAMIDES IN HOMOGENATES OF MEAT FOR BABIES**

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The sulfonamides (SFAs) are synthetic antibiotics too widely used in veterinary practice for therapeutic, prophylactic or growth-promoting purposes. As consequence, problems associated with residues of sulfonamides in animal source foods include the risk of adverse health effects, enhanced resistance of pathogenic bacteria towards antibiotics activity, but also the increase of allergic and carcinogenicity effects. The analytical determination of such residues in meat and other animal source foods used for human consumption has become an important task. Thus, sensitive, selective, and economic analytical methods for the determination of these molecules in food matrices, are needed. Several analytical methods based on liquid chromatography (LC) coupled with spectrophotometric detectors or with mass spectrometry have been proposed to determine SFAs in various real matrices (1-2). However, to our knowledge, no selective method for the determination of SFAs, based on the LC technique combined with the pulsed amperometric detection (PAD) on noble metal electrode substrate has been reported. The aim of this work is to investigate the potentiality of the electrochemical detection of these molecules.

A liquid chromatography methodology based on the C<sub>18</sub> reverse phase for the simultaneous separation of nine sulfonamides, was studied and optimized. The amperometric detector using a polycrystalline gold substrate as working electrode and operating under pulsed amperometric detection mode (PAD) in neutral buffered phosphate medium at pH 7 was tested for the determination of the selected molecules. Under optimal chromatographic and amperometric conditions, the limit of detection of the investigated sulfonamides are comprised between 1  $\mu$ M and 0.05  $\mu$ M and the dynamic linear range spanned generally over three orders of magnitude. A liquid extraction procedure based on the use of acetonitrile solvent is studied for the quantitative extraction of sulfonamides from homogenized of meat for babies. These molecules are successful separated and analyzed by LC technique coupled with a PAD scheme.

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# A NEW MICRO LIQUID/LIQUID EXTRACTION, ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-QUADRUPOLE/TIME OF FLIGHT MASS SPECTROMETRY METHOD FOR THE CHARACTERIZATION OF PHENOLIC FRACTION OF OLIVE OIL

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Extra virgin olive oil (EVOO) is a valuable component of the traditional Mediterranean diet, unique among other vegetable oils for its fatty acid composition and its high concentration level of phenolic compounds. The phenolic fraction of EVOO consists of a heterogeneous and very complex mixture of compounds, each having a particular influence on the quality of EVOO (1). Among them, phenolic compounds could play a major role for human health because some of their marked antioxidant, antimutagenic, cardioprotective, antimicrobial, and antiviral properties (2,3). In order to trace a complete profile of these compounds, we developed a new method for their extraction based on the use of liquid extraction/partition and dispersive solid phase extraction (QuEChERS: Quick, Easy, Cheap, Effective, Rugged, and Safe) consisting in a selective micro liquid/liquid extraction (LLE) of target compounds, followed by a rapid purification utilizing C<sub>18</sub> as dispersing material. A factorial experimental design was used for method optimization. Oil sample (500 µg) was placed in a polycarbonate tube and added with 500 µL hexane and 2 mL methanol, and extracted by shaking for about 3 min. After centrifugation for 3 min at 3000 rpm, the alcoholic phase was recovered in a clean tube, and 50 mg C<sub>18</sub> added. Polycarbonate tube was shaken for 1 min and centrifuged for another 1 min at 3000 rpm. Supernatant was forced through a PTFE syringe filter (0.2 µm), and the filtrate was then evaporated to dryness under a stream of nitrogen. The residue was reconstituted with 300 µL of methanol/water (60:40, v/v) 0.1% HCOOH. The isoflavone biochanin A, absent in olive, was used as internal standard. Two µL of the final solution was injected into the system, consisting of an UHPLC system coupled to ESI Q-TOF MS/MS. As this analytical technique was applied by the first time to the oil matrix, a comparison between the performances of other techniques more widely used, such as SPE and matrix solid phase dispersion extraction methodologies respect to micro LLE, was performed.

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## DEVELOPMENT OF A MULTICLASS METHODOLOGY FOR EXTRACTION AND LC-MS/MS DETERMINATION OF VETERINARY DRUGS AND MYCOTOXINS IN EGG

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Antibiotics such as tetracyclines and coccidiostats are commonly employed in chicken farms to prevent bacterial infections and coccidiosis; other drugs, such as fluorquinolones and sulfonamides, are administered to hens as growth promoters, although their use is not allowed in European Union (EU). Mycotoxins produced by different filamentous fungi (mainly *Penicillium*, *Fusarium* and *Aspergillus*) can reach animals through contaminated feed, especially if not properly stored. All these contaminants can be transmitted from animal to derived-food such as eggs. Indeed, EU has settled maximum limits (MLs) for many veterinary drugs (1) and mycotoxins in eggs (2).

A QuEChERS (Quick Easy Cheap Effective Rugged Safe) extraction method has been developed for simultaneous analysis of veterinary drugs and mycotoxins in hen eggs by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with electrospray (ESI) source. Different classes of both antibiotics (tetracyclines, ionophores, penicillins, cephalosporins, fluoroquinolones, sulfonamides) and mycotoxins (enniatins, ochratoxins, aflatoxins) have been considered for method development. Particular attention has been devoted to extraction optimization: different solvents (acetone, methanol, hydroalcoholic solutions), different pH values and different salts (such as anhydrous Na<sub>2</sub>SO<sub>4</sub>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and NaCl, fundamental in QuEChERS methods to enhance phase separation) have been tested and evaluated in terms of recovery, relative standard deviation (RSD) and ESI signal suppression due to matrix effect.

Both chromatographic and mass spectrometric conditions have been optimized to obtain the best instrumental performances for most of the analytes. Quantitative analysis has been performed by means of matrix-matched calibration, in the range 10-100 µg/kg depending on the analyte and its ML. Recoveries ranged between 70-80% (2-25% RSD) for all the analytes but doxytetracycline (36±13%) and lasalocid A (37±10%). The multiclass method proposed is rapid, simple, and allows low solvent consumption, in line with QuEChERS features and modern analytical requirements.

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## GLUCOSINOLATE PROFILE OF *BARBAREA VULGARIS* SEEDS EVALUATED BY LC-ESI-FTICR MASS SPECTROMETRY

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An important group of bioactive components occurring in *Barbarea vulgaris* seeds are glucosinolates (GLSs), which are sulfur-rich plant secondary metabolites (1). *B. vulgaris* is a vegetable belonging to *Brassicaceae* family and considered as a food supplement. Liquid chromatography with electrospray ionization coupled with Fourier-transform ion cyclotron resonance mass spectrometry (LC-ESI-FTICR MS) and tandem MS, performed by infrared multiphoton dissociation (IRMPD) in the high-resolution trapping cell, were employed to identify GLSs and their derivatives in extracts of *B. vulgaris* seeds. Along with most common GLSs and derivatives already found in *B. vulgaris* seeds such as glucoarabihirsuin, glucobarbarin, glucobrassicin, gluconasturtiin, 6'-isoferuloyl-glucoarabarin, 6'-isoferuloyl-glucoarabarin and 6'-isoferuloyl-gluconasturtiin (2), the occurrence of the uncommon 6'-cumaroyl-glucoarabarin and 6'-dimethoxycinnamoyl-glucoarabarin is reported. Besides, the presence of five additional GLSs, *viz.* glucocapparin, sinigrin, glucoelongatin, glucoarabihirin, glucoerucin, was found for the first time. On the basis of their fragmentation behavior, all compounds were successfully identified with mass errors lower than +1.7 ppm. All GLSs and derivative-GLSs were characterized by several product ions, including the HSO<sub>4</sub><sup>-</sup> ion at *m/z* 96.96012, being a good clue for a compound belonging to the GLS family. Moreover, typical fragments derived from thioglucoside moiety and from the side-chain were observed (3,4). It was confirmed that the profiles of isoferuloyl derivatives matched the profiles of non-acylated GLSs (2).

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## LC/MS/MS DETECTION OF SHORT-CHAIN AMINES AND MORPHOLINE IN WAX FORMULATIONS FOR FRUIT COATING

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An analytical protocol was developed for investigating short-chain aliphatic amines occurrence in waxes for fruit coating with the aim of commercialization control in countries where the use of these carriers are forbidden. Morpholine, diethanolamine, triethanolamine, triethylamine, 2-dimethylaminoethanol and 3-metoxypopylamine were detected by LC/MS<sup>n</sup> operating in positive ion mode in fortified waxes after a two step clean-up procedure. Despite a large number of analytical determinations of aliphatic amines through mass spectrometry detection has been done, the most applications have been performed on environmental matrixes. Amine studies on food samples have been carried out but no application of HPLC-MS has been made up for amines determination in food waxes. The most significant improvement of the LC/ESI-MS<sup>n</sup> method employed, respect to common analytical approaches such as gas and ion chromatography with or without derivatization (1, 2, 3), was the possibility of avoiding time consuming preparative purification step, facilitating the sample handling and decreasing the analysis time and the solvent consuming. The reverse phase separation coupled with MS<sup>n</sup> detection allowed the sure identification of the amines reducing the strong matrix effect of a complex samples like coating waxes. The sensitivity, the precision and the accuracy make the method adequate for quantification in glazing agents employed in fruit coating. These characteristics lend the method suitable for control regulatory agency applications. The method was statistically validated. The matrix matched regression lines showed  $r^2 > 0.97$ . Recoveries ranging from 92 to 114% were obtained for the fortification level of 3.5% w/w and the relative standard deviations ranged from 2 to 8% (n = 6). The limits of detection were below 0.2% w/w, while the limits of quantification did not exceed 0.5 % w/w (4).

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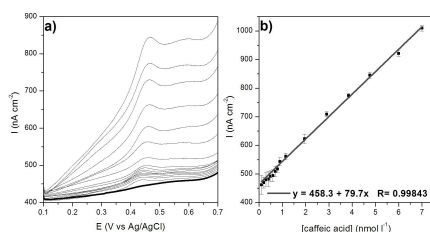
## DETERMINATION OF CAFFEIC ACID IN WINE AT POLY(3,4-ETHYLENEDIOXY)THIOPHENE PLATINUM MODIFIED ELECTRODE: A PRELIMINARY STUDY

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Phenolic compounds are a class of naturally occurring compounds widely distributed in nature, which are essential for the growth and reproduction of plants as well as for protection against pathogens [1, 2]. Belonging to this group, phenolic acids are structurally related possessing one carboxylic acid functionality, at least. Besides, they can be subdivided into two main groups: benzoic acid derivatives (e.g., gallic acid) cinnamic acid derivatives (e.g., caffeic acid and chlorogenic acid) and flavonoids (e.g., catechin, rutin). Among cinnamic acid derivatives, caffeic acid, (CA), 3,4-dihydroxycinnamic acid, is one of the most investigated not only because of its protective antioxidant behaviour but also due to its action against immunoregulation diseases, asthma and allergic reactions. As a consequence, studies involving the development of analytical methodologies for the detection and measurement of caffeic acid and its derivatives from plant sources, human fluids and several beverage products as well have been increased. A method using PEDOT (poly(3,4-ethylenedioxy)thiophene) modified electrode was developed for evaluation of caffeic acid (CA) in wine. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used for measurement of this analyte by means of PEDOT modified electrodes. PEDOT films were electrodeposited on platinum electrode (Pt) in aqueous medium by galvanostatic method using as electrolyte and surfactant sodium poly(styrene-4-sulfonate) (PSS). CV allows to detect the analyte over a wide concentration range ( $10 \text{ nM}^{-1}$ - $6.5 \cdot 10^{-3} \text{ nM}$ ) and by means of DPV (linear range 0.1 nM-10 nM) it was further possible to decrease the LOD (from 3 nM to 0.1 nM).



**Fig. 1.** a) DPV @ Pt-PEDOT-PSS modified electrode for different concentration of caffeic acid in wine model solution at pH 3.6. b) Calibration curve of caffeic acid. Concentration range: 0.1 nM to 7 nM.

## COMPARISON OF DIFFERENT COCOA LIQUORS BY GC-MS AND LC-MS/MS

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Cocoa and chocolate are among the most appreciated food worldwide. Their characteristic flavors are due to a complex volatile fraction whose composition depends both on the cocoa bean genotype and the several processes occurring in the chocolate production (fermentation, drying, roasting and conching).<sup>[1]</sup>

Among the different volatile compounds in food, the class of pyrazines is one of the most studied:<sup>[2]</sup> they constitute a significant portion of the substances formed during roasting and for this reason they can be used as index compounds for the roasting process<sup>[2,3]</sup> and could be one of the useful parameters for the optimization of the whole chocolate production.

In this work, a headspace solid-phase microextraction gas chromatography-mass spectrometry method was developed for the qualitative study of the volatile fraction in five cocoa liquors with different geographical origins. The quantitative determination of five pyrazines (2-methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,3,5-trimethylpyrazine and tetramethylpyrazine) was performed by the standard addition method. Good figures of merit were obtained; in particular limits of quantitation were in the range 0.1-2.7 ng/g. Tetramethylpyrazine showed the highest concentration in all samples, with a maximum value of 585 ng/g. The considered samples differed in total pyrazine content, ranging from 99 to 708 ng/g.

A preliminary study for the characterization of the non-volatile fraction by LC-MS/MS was also performed, identifying some flavanols such as catechin, epicatechin and procyanidins.

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**IDENTIFICATION OF A COUNTERFEIT NUTRITIONAL SUPPLEMENTS ADULTERATED WITH THE ANTI-INFLAMMATORY DRUG NIMESULIDE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY - DIODE ARRAY DETECTION (HPLC-DAD) AND ATTENUATED TOTAL REFLECTANCE (ATR-FT-IR)**

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Food and consumer product fraud, or adulteration and counterfeiting, is increasingly a critical problem for the food and consumer products industries. In this work we report a case of counterfeiting of a popular nutritional supplement (PC-28) and its adulteration with nimesulide (NIME), a well known prescription drug with anti-inflammatory activity which presence, as well as that of any other kind of drug, is strictly forbidden in such kind of products. Different batches (years 2006-2010) of the nutritional supplement were collected directly from the producer or in a pharmacy store in Milan. A second set of batches seized by Italian authorities was analyzed by an independent laboratory. HPLC-DAD analysis of acetonitrile extracts of the tablets, indicated three different groups: (i) batches free from NIME (starting from 2008), (ii) batches with NIME in trace amount (25-79  $\mu\text{g}/\text{tablet}$ , produced from 2006 to 2008), (iii) batches heavily contaminated with NIME (25-95  $\text{mg}/\text{tablet}$ , all produced in 2008), in amounts that allowed the detection of the drug by direct analysis of the tablets by ATR-FT-IR. Some of the batches in (ii) and (iii) had the same batch code. The low level of contamination of batches in group (ii) was explained by the undeclared NIME content of one of the active ingredients (imported from China) used for their production (5.6% w/w), taking into account of the dilution with the other active ingredients and excipients. In the case of one of the batches belonging to (iii), the careful visual examination of the blister containing the tablets in combination with their ATR-FT analysis revealed that the macroscopic (blister forming, shape, symmetry and deepness) and microscopic (type of polymer and ink) characteristics were not compliant with those typical of the producer company, demonstrating that these samples were counterfeit copies of those in group (iii).

## **SOLID PHASE EXTRACTION OF PENICILLINS FROM MILK THROUGH THE USE OF SACRIFICIAL SILICA BEADS AS SUPPORT FOR MOLECULAR IMPRINTING**

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Molecularly imprinted polymer has been increasingly used for the solid phase extraction of penicillins from food samples. Notwithstanding this approach is successful, the presence of residual template in the imprinted polymer and the difficulty to achieve selectivity focused on structurally related compounds represent significant drawbacks. The goal of this work was to prepare molecularly imprinted beads with molecular recognition towards target molecules characterized by the presence of a penicillanic sub-structure, and to use them to set-up a solid phase extraction protocol compatible with a micellar electrokinetic chromatography for the analysis penicillins in skimmed milk. An imprinted polymer was prepared by filling the pores of mesoporous silica beads — previously grafted with 6-aminopenicillanic acid as mimic template — with a 2+3 mixture of methacrylic acid and trimethylolpropane trimethacrylate, polymerizing it and dissolving the inorganic support by corrosion with ammonium fluoride. The imprinted beads showed good molecular recognition properties for target molecules presenting the penicillanic group as common structural feature, while other antibiotic molecules such as cephalosporins or chloramphenicol were poorly recognized. They were used to efficiently extract penicillins (penicillin V, nafcillin, oxacillin, cloxacillin and dicloxacillin) from skimmed and deproteinized milk in the concentration range of 5-100  $\mu\text{g/l}$ . The extracts were analysed by micellar electrokinetic chromatography in TRIS-borate buffer, 200 mM, 30 mM SDS, pH 8 applying a reverse polarity staking as in-capillary pre-concentration step, resulting in a fast and affordable method with minimum pre-treatment of the milk samples and recovery levels of 64-90%.

## SCREENING OF A COMBINATORIAL LIBRARY OF ORGANIC POLYMERS FOR THE SOLID PHASE EXTRACTION OF PATULIN FROM APPLE JUICE

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Patulin (4-hydroxy-4H-furo[3,2c]pyran-2[6H]-one) is a water-soluble mycotoxin produced by several *Aspergillus* and *Penicillium* species of fungi which presence is notably considered common in apples. It has come under scrutiny for its potential negative health effects because of its suspected mutagenic, teratogenic and carcinogenic activity. Of consequence, several governmental bodies have posed maximum residue limits for this mycotoxin. However, the direct detection of patulin in complex food matrices can be a difficult task, and sample clean-up treatments are frequently necessary before performing the instrumental analysis..

With the aim of simplifying the clean-up step, in recent years there has been some efforts to prepare highly selective artificial materials for the extraction of mycotoxins from complex samples. Beside molecularly imprinted materials where the template is represented by the target mycotoxin, several papers have recently been published about polymers prepared without the use of a template molecule but characterized by good selectivity and binding properties towards the target compounds. As these papers are based on the virtual screening of *in silico* libraries of monomeric precursors, it is plausible that the same results could be obtained by directly screening a library of organic polymers characterized by an high degree of molecular diversity. In this work we prepared a 256-member combinatorial polymeric library based on 16 functional monomers, 4 cross-linkers and 4 different porogenic solvents. This library was screened for the binding with patulin in different environments such an acidic buffer and acetonitrile. The polymer with the best binding properties was used to develop a solid phase extraction material for the extraction of patulin from apple juice. Satisfactory sample clean-up for apple juice spiked at 10, 25 and 50 ng ml<sup>-1</sup> of patulin was achieved by the extraction protocol and recoveries came out at 64±2.5% at 10 ng ml<sup>-1</sup>, 83±2.6% at 25 ng ml<sup>-1</sup> and 76±2.5% at 50 ng ml<sup>-1</sup>, thus reproducible and in good agreement with the recoveries performed on buffer alone.

## THE EXTRACTOR NAVIGLIO® IN FOOD PRODUCTIONS

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For over a decade the Extractor Naviglio has been a good alternative to solid-liquid extraction techniques such as maceration and percolation (1). That has been broadly demonstrated since the extractor provides the same quality or even higher of the extracts obtained by the traditional extraction techniques, with a significant reduction in the duration of the extraction process (ten days of maceration extraction correspond to about one hour of extraction by the Extractor Naviglio under the same extractive conditions) and a more efficient extraction (2,3). The principle on which it works (Naviglio's Principle) (4) is studied in graduate courses in Herbal Techniques of several Italian Universities. Currently the Extractor Naviglio is widely used in many fields of research and production (herbals, nutritional supplements, cosmetics, beverages etc.). In the food sector, in particular, the Extractor Naviglio has been shown to be a viable alternative to maceration for: production of lemon liquor (limoncello) and similar liquors; production of bitters and elixir of juniper; rapid aging of wines, brandies and distilled liquors; extraction of lycopene from tomato processing waste (2,3,5,6). Recently, more unconventional applications of the Extractor Naviglio have been studied, as the rapid rehydration of legumes and their simultaneous aromatization, cleaning of washers for the production of cork stoppers, cleaning of rubber polymers, tanning of leather.

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## FOOD QUALITY CONTROL: APPLICATION OF NEAR INFRARED SPECTROSCOPY FOR DRIED EGG-PASTA CHARACTERIZATION

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Food quality control is not an optional extra in food processing; neither is it something done only by large manufacturers. It is an essential component of any food processing business. Quality control need not be time consuming or expensive, and the results of quality control tests should help save money in the long run. In general, quality control procedures should be as simple as possible and only give the required amount of information. Quality control is used to predict the quality of the processed food and then control the process so that the expected quality is achieved for every batch. This means that quality specifications must be written and agreed with suppliers or sellers, and control points must be identified in the process. Today pasta has become a dietary staple all over the world. Dried egg-pasta is important in the market, since the range of about 50 different dried egg pasta shapes reflect traditional regional Italian cuisine. The success is due to the unique characteristic that dried egg-pasta looks and tastes like home made and is available in many unusual shapes and sizes. When considering dried egg-pasta, three are the main parameters which can affect the quality of the final product, drying time and temperature, and the amount of eggs used. Indeed, on one hand, thermal processes, have an influence on the quality of pasta on a macromolecular level due to reciprocal interactions between proteins and starch. In particular, changes in dried and in cooked pasta structure were determined regarding protein solubility, thermal properties and digestibility of starch, microscopic and rheological measurements. On the other hand, the color, taste, flavor, texture and cooking properties of different dry pasta products are determined primarily, besides the quality of ingredients used, by the quantity of eggs added.

Based on these considerations, in this study the possibility of using NIR spectroscopy as a rapid and non destructive tool to assess dried egg pasta quality was investigated, by determining the influence of the three main parameters (egg percent amount, drying temperature and drying time) on the spectroscopic fingerprint of the final product. Reference pasta samples were prepared with different egg percent concentration (20%, 22%, 25%, 27%, 30% and 33%), and different drying temperatures and times were tested. The results show that all of the three parameters have a relevant impact on the shape of the spectroscopic signal. Therefore, NIR spectroscopy appears a very promising tool to be applied *at-line* in pasta industry since it is able to monitor the modifications induced by the change of each considered parameter. A similar approach has never been reported in the literature, where only one study can be found and it is simply voted to the egg percent determination of few commercial samples.

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## **A RAPID HPLC METHOD FOR THE DETERMINATION OF FREE HMF IN ROYAL JELLY**

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Royal jelly (RJ) constitutes mostly the exclusive food for the queen honey bee throughout her life. It is secreted from the hypopharyngeal glands of the young workers and it plays a key role for the caste differentiation inside the beehive. Chemical composition of RJ changes in relation to bee species, season of production and geographical origin and it mainly consists of water, proteins, sugars, lipids and mineral salts. Minority fraction of RJ includes water-soluble vitamins, nucleotides, organic acids, acetylcholine and other bioactive and nutraceutical substances. Freshness represents one of the most important aspects related to royal jelly quality. Improper storage causes changes in the physical and chemical features and may determine a loss of its nutraceutical properties and, consequently, of its commercial value. Many of the physical and chemical modifications that royal jelly goes through during storage can be attributed to the Maillard reaction. In particular, 5-hydroxymethyl-2-furadehyde (HMF) is one of the chief main products of such reaction and represents a well-known freshness parameter for honey. On the other hand, the chemical composition of RJ suggests that many of the pathways involved in HMF formation in honey may be active also in such foodstuff. Hence, pursuing our interest (1,2) in the assessment and validation of new analytical methods for the determination of minority species for beehive products, we propose a fully-validated, original analytical RP-HPLC procedure aimed to measure free HMF in royal jelly. Also the first results by the application of this method on a number of well and inadequately stored real samples of RJ are here reported.

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## THE ROLE OF IRRIGATION TECHNIQUES IN ARSENIC BIOACCUMULATION IN RICE (*ORYZA SATIVA L.*)

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The bioaccumulation of arsenic compounds in rice is of great concern worldwide because rice is the staple food for billions of people and arsenic is one of the most toxic and carcinogenic elements at even trace amounts. The uptake of arsenic compounds in rice comes mainly from its interaction with system soil/water in the reducing conditions typical of paddy fields and is influenced by the irrigation used. We demonstrate that the use of sprinkler irrigation produces rice kernels with a concentration of total arsenic about fifty times lower when compared to rice grown under continuous flooding irrigation. The average total amount of arsenic, measured by a fully validated ICP-MS method, in 37 rice grain genotypes grown with sprinkler irrigation was  $2.8 \pm 2.5 \mu\text{g kg}^{-1}$  whereas the average amount measured in the same genotypes grown under identical conditions, but using continuous flooding irrigation was  $160 \pm 20 \mu\text{g kg}^{-1}$ . In addition, we find that the average concentration of total arsenic in rice grains cultivated under sprinkler irrigation is close to the total arsenic concentration found in irrigation waters. Our results suggest that, in our experimental conditions, the natural bioaccumulation of this element in rice grains may be completely circumvented by adopting an appropriate irrigation technique.

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## **SORCE APPORTIONMENT STUDY NEAR A MSW INCINERATOR BY POSITIVE MATRIX FACTORIZATION (PMF)**

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Chemometric methods are very spread and useful in data analysis of complex dataset, since they allow to reduce the number of factors that explain a phenomenon and make the study easier to interpret. These methods are particularly important in environmental studies. They allow to colleague contaminants related to the same source and to undertake source apportionment studies. Indeed, in order to reduce the amount of pollutants in atmosphere, it is important to determine the contribution given by the different emission source (1). Positive Matrix factorization (PMF) is a new approach compared to Principal Component Analysis (PCA) and it has several advantages for the applications in environmental studies. First of all measure uncertainties and below detection limit data can be managed. But the most important characteristic is that loadings matrix has only positive values and this is a fundamental feature in source apportionment studies, where each factor should represent a different emission source (2, 3).

The aim of this study is to evaluate the contribution of Coriano (RN) MSW incinerator and of other emission sources in the studied area. To obtain that, heavy metals and soluble ions load, in wet and dry deposition fluxes, have been determined and PMF analysis was applied. From 2006 to 2010, bulk atmospheric depositions of inorganic contaminants have been determinate monthly in 4 sampling sites. These have been situated in areas differently affected by plant emissions fallouts, according to the results of the Calpuff air dispersion model. The sources affecting the area, other than the incinerator, are the coastal city of Riccione (vehicular traffic and domestic heating) and the A14 highway.

Results show that the studied area is subject to low contamination, as far as these compounds are concerned. Deposition flows do not show a greater amount of contaminants at sites most influenced by plant emissions fallouts. PMF analysis indicates that the contribution of heavy metals is especially due to soil sources. In conclusion, the incineration plant is not the main source of inorganic pollutants in the studied area, which is characterized by a homogeneous contamination, typical of an urban area.

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## A CASE OF MANAGEMENT OF A TOXIC CYANOBACTERIUM BLOOM (*PLANKTOTHRIX RUBESCENS*) AFFECTING AN ITALIAN DRINKING WATER BASIN

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An extraordinary bloom of *Planktothrix rubescens* was observed in the early 2009 in the Occhito basin (1), used even as source of drinking water in Southern Italy. A task force involving the main stakeholders was created to face this environmental and sanitary emergency with short, medium and long term corrective actions. Over two years, a sequence of activities aimed to assess, manage, mitigate and communicate the risk associated to drinking water contaminated by cyanobacteria were implemented. The main products were: development of analytical protocols, monitoring of the entire water chain, solutions for the water treatment process, training of operators, a dedicated website. A liquid chromatography (LC)-tandem mass spectrometric (MS) method for the analysis of 9 microcystins (MCs) was optimized and also used to check the reliability of the ELISA method, sometimes evidencing an uncommon underestimation. MC-LF and MC-LY were detected for the first time in Italian freshwaters. Several relevant water samples were analyzed with the high resolution MS, using a LC-QToF system, with the aim to identify other potential cyanotoxins that are not determinable without available analytical standards. Preliminary results has shown the presence of other uncommon cyanotoxins in Italy. The efficiency of the processes used by the Water Treatment Plant of Finocchito have been evaluated in terms of reduction of algal cells and toxins and improved with Granular Activated Carbon filters. The pre-oxidation with chlorine dioxide followed by the flocculation and settling have been shown to be effective in removing MCs. Despite the high levels of cyanobacteria (up to  $160 \times 10^6$  cells/L) and MCs (28.4  $\mu\text{g/L}$ ) initially reached in raw surface water, the drinking water distribution was never limited, because the concentration of MCs was always below the WHO guideline value.

This experience of a drinking water chain monitoring for cyanobacteria and cyanotoxins was the main "case study" used for the compilation of the Italian Guidelines for risk management of Cyanobacteria in water for human consumption (2).

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## **CHARACTERIZATION OF THE CHEMICO-PHYSICAL AND HYDROGEOLOGICAL PARAMETERS OF SPRING WATERS IN THE AREA OF LIVIGNO (ITALY)**

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The investigation of the chemical composition of spring waters is of fundamental relevance for understanding both the circulation pathways and the exploitation possibilities. Such studies acquire particular importance when the needs of mountain communities are involved. For these purposes, an exhaustive study was carried out on several Alpine springs on the territory of Livigno (Sondrio, Italy). During a three year monitoring, we have sampled and analysed 52 springs for chemico-physical and hydrogeological parameters. In particular, pH, temperature and conductivity were determined on site, whereas major and trace components were analysed by ion chromatography and Inductively Coupled Plasma – Mass Spectrometry, respectively. Moreover, a portable monitoring system, constituted by a weather station and a multi-parametric probe powered by a solar panel, was used to obtain data with high temporal resolution in the field. The results were elaborated by means of Piper and Schoeller diagrams, while basic theme maps were created using different kinds of data: field data, chemical analysis data, hydrogeological measurements and structural geological data. As a result, the springs were classified according to their chemical composition: the correlations with the lithologic composition enabled the understanding of the underground water circuits.

## SPECTRAL MODIFICATIONS OF LAKE AND SUBTERRANEAN WATERS UNDER IRRADIATION

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The effects that sunlight exposure may have on the properties and photochemical behaviour of chromophoric dissolved organic matter (CDOM) was studied. Water samples from subterranean systems and from lake epilimnion were optically characterised and irradiated under simulated sunlight. Irradiation of CDOM by sunlight causes both a decrease of the absorbance (photobleaching) (1), and mineralization (4-6). Interestingly, it has been found that groundwater CDOM is much more susceptible to photomineralization than CDOM in lake water. (7).

The studied subterranean water samples were quite different from lake water ones, concerning specific absorbance, EEM spectra, and absorption spectral shape. Differently from lakes, absorption spectra of subterranean water samples showed variations from the typically observed, featureless exponential decay of absorbance *vs.* wavelength. With one exception, subterranean water had a higher proportion of aquagenic/autochthonous CDOM (*e.g.* proteinaceous material) compared to pedogenic/allochthonous one (*e.g.* humic and fulvic substances). Quite interestingly, irradiation of subterranean water produced very significant spectral changes, and finally yielded lake water-like exponential absorption spectra. In contrast, irradiation of lake water produced photobleaching, but the shapes of the absorption spectra underwent rather limited variations. This finding suggests that exposure of CDOM to sunlight may play a key role in shaping the absorption spectra of surface waters. In fact, non-exponentially absorbing CDOM would be quickly transformed into exponentially-absorbing substances.

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

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

The SPR-based biosensors approach relied on the inhibition of the hybridization of selected oligonucleotides after formation of the DNA adduct. Quantitative information as well as difference in the kinetics has been observed and characterize for different oligonucleotides.

These bioanalytical assays could be used for the detection of potential genotoxicity of different chemicals.

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## AUTHENTICITY ASSESSMENT OF COMMERCIAL OLIVES IN BRINE

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Analytical methods for confirmation of food authenticity claims should be rapid, economic, non-destructive and should not require highly skilled personnel for their deployment [1]. An ideal method should also give a response independent of the particular equipment used. In the present study, near-infrared (NIR) spectroscopy was used for verifying authenticity of commercial olives in brine of cultivar Taggiasca produced in Liguria.

39 samples, representative of the 2011 production of Taggiasca olives in brine, were analysed together with 81 samples produced with olives of other cultivars, morphologically similar to the Taggiasca one (i.e. Leccino and Coquillo). All of the samples were collected directly from certified producers, by the chemical laboratory of the Special Company for Professional Training and Technological and Commercial Promotion of the Chamber of Commerce of Savona.

NIR spectra were acquired in two laboratories with two different FT-NIR spectrophotometers: an AntarisII (Thermo Scientific, the master instrument) and a Nirflex N-500 (Büchi, the slave instrument).

A mathematical spectral transfer correction was deployed to minimise the systematic differences existing between signals recorded with the two instruments, that are not constant along the whole spectral range. The regression of the average spectrum of the slave instrument against the average spectrum of the master instrument is computed piecewise, by means of a moving window. The regression coefficients computed are then used to correct spectra obtained by the slave instrument, making them comparable with those recorded on the master instrument. This allows a unique class model, developed on master instrument, to be applied for authenticity verifications on samples, independently of the laboratory in which they are analysed.

Class models for the verification of olive authenticity were built by the unequal dispersed classes (UNEQ) method [2], after data compression by disjoint principal component analysis (PCA). Models were validated on an external test set.

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## EFFICIENT STRATEGIES FOR DATA COMPRESSION OF HYPERSPECTRAL IMAGES

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HyperSpectral Imaging (HSI), also known as chemical or spectroscopic imaging, represents an emerging technique that provides both spatial information proper of imaging methods and spectral information typical of spectroscopy (1). Compared with traditional spectroscopic techniques, HSI allows one to acquire spectral data in correspondence to each pixel of an image. It also enables the visualization of the chemical composition of the samples, retaining at the same time the advantages of being fast, non-destructive and of not requiring chemicals. Unlike RGB images, hyperspectral images are made up of more than one hundred of congruent images recorded at different wavelengths and stacked to each other, forming a three-dimensional (3D) matrix, or hypercube, comprising two spatial (x and y) and one spectral ( $\lambda$ ) dimensions. However, in order to properly exploit the great potentialities offered by this technique, efficient chemometric methods must be developed in order to extract the useful information contained in such a wide amount of data. The compression of useful information into an optimized set of few parameter values, in fact, is essential for analysing datasets formed by a large number of images, as well as for enabling on-line monitoring.

In this context, we propose a strategy to reduce significantly the required computational load and time. This procedure is derived from the *colourgrams* approach, already developed by some of us for the elaboration of RGB images (2). It essentially consists in compressing the useful information contained in each hypercube into a signal, named *hyperspectrogram*, which is composed by the frequency distribution curves of quantities calculated by PCA. Hyperspectrograms can then be used as a compact set of descriptors and subjected to further blind analysis techniques. Moreover, by applying proper variable selection methods, the data can be further compressed and the interpretation of the results in chemical terms is also made possible, through the visualisation of the selected features in the form of an image.

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## CHEMOMETRIC METHODS FOR THE DEVELOPMENT OF A NEW SENSING SYSTEM FOR THE IDENTIFICATION OF DRUG PRECURSORS IN GASEOUS PHASE

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In order to efficiently detect four selected drug precursor molecules in presence of interfering species and background air, a modulated laser source with photoacoustic detection, operating in the mid-infrared region has been realised. A complex strategy of simulation of spectral responses has been developed by collecting spectra of gases from the literature. The spectra have been denoised by means of Wavelet Transform (WT) (1) and mixed together according to a concentration matrix. This was specifically built using Experimental Design techniques to represent a comprehensive combination of possible realistic cases. In order to scale the database spectra to the proper concentration levels, an ad-hoc algorithm based on a sigmoidal transfer function was also implemented, capable both to preserve the baseline shape and to fit correctly the peak intensities. Subsequently, by means of the same WT-based method and of an algorithm developed by following previous literature suggestions (2), a preselection of the spectral range has been made. Care was taken in order to avoid considering noisy regions due to small molecules present as pollutants or air components. Suitable instrumental noise was finally added, simulating the noise structure evaluated from preliminary experimental measurements made with the realised instrumental system. In this way, 2000 spectra were built, representing a wide variety of gas mixtures. This spectral dataset was then used to select the optimal 200  $\text{cm}^{-1}$  wavenumber range, by maximizing the classification efficiency estimated by PLS-DA (3) in cross-validation, on a moving window. Finally, the optimal wavenumber values were identified using a newly developed interface, based on Genetic Algorithms (4) and on resampling of mixtures spectra.

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## NEW NONLINEAR PERSPECTIVES IN CHEMOMETRICS: THE ATDM METHOD

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When dealing with analytical problems and search for quantitative relationships between molecular structure and biological activities (QSAR), complex data systems are currently encountered, which need proper methods to be analyzed. These data systems naturally contain diverse sources of information and data patterns. The most common linear methods of multivariate analysis, as, for instance, principal component analysis (PCA), multidimensional scaling (MDS) and cluster analysis, are frequently able to discover only a few relationships among data, neglecting the non-linear part of information and resulting in conclusions that are incomplete and in some cases misleading.

*Atemporal Target Diffusion Model* (ATDM) is a recently proposed algorithm<sup>1</sup> that has been developed to detect the dependencies among pairs of variables in large datasets, whilst also taking approximate account of their higher order relationships with other variables. The results of an ATDM analysis are given in terms of a minimum spanning tree (MST), which allows one to have simple graphical visualization and inspection of data relationships and patterns.

Performance of the ATDM method was investigated and discussed towards the most classical methods of data analysis by means of some datasets targeted to exploratory data analysis.

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## **EVALUATION OF THE PERFORMANCE OF MALDI-TOF FOR QUANTITATIVE AND SEMIQUANTITATIVE DETERMINATIONS THROUGH MULTIVARITE TOOLS**

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MALDI-TOF is widely applied in the field of proteomics both for the identification of proteins from digests of 2D-PAGE spots and for the characterization of complex protein mixtures. In this last case, MALDI-TOF is applied to provide mass spectra that can be used for the identification of candidate biomarkers between control and pathological and/or treated samples. In other cases, MALDI-TOF spectra are used for identifying differences in spectra due to increasing doses of a drug (clinical proteomics) or a pesticide (environmental proteomics). Moreover, MALDI-TOF analysis is usually characterized by a low reproducibility that hampers its use in biomarker identification studies: the variability involves also the use of different positions on the MALDI Plate and the sampling position selected on each spot of the plate.

From these starting considerations, we evaluated the potentials of MALDI-TOF in (semi)quantitative analyses through a study of the variability of the measurements recorded on a standard sample. Three experimental factors underwent analysis: the laser intensity, the number of shots to be collected, the varying concentration of the standard. These three factors were studied at three levels. Three different plates were run on different days and the effect of the position on the plate was investigated by randomizing the sample location on the plate. Each experimental condition (laser intensity, number of shots and concentration) was replicated for variability evaluation. The acquisition system allowed the collection of a maximum of 9 points on each single spot: these measurements were used to investigate the homogeneity of sample deposition within each spot.

## CLASSIFICATION MODELS IN NEWBORN SCREENING FOR METABOLIC DISEASES BY LC-MS/MS

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Metabolic diseases are caused by an absence or a deficiency of an enzyme deputed to intracellular production of energy in the body. Newborn screening of metabolic diseases can highlight a biochemical change in the first stage of sickness. The use of tandem mass spectrometry (MS/MS) to detect metabolic disorders in newborn is one of the most important advancement in clinical screening technology, as it offers to screen “one spot, one test, many diseases” at the same time (1). The aim of this work was to identify possible relationships between biomarkers and different diseases by applying unsupervised methods, such as Principal Component Analysis (PCA) (2), and supervised classification methods, like Linear and Quadratic Discriminant Analysis (LDA, QDA) (3) and Classification And Regression Trees (CART) (3). 4625 samples of healthy and non-healthy infants, each characterized by 39 descriptors (amino acids, fatty and organic acids), tested over the past 5 years by LC-MS/MS, were used to find a classification model. Prior to classification, data were divided into a training (3236) and a test set (1389) using the duplex algorithm (4). A comparison of the different classification models was made on the basis of their predictive ability, simplicity, and interpretability. For the classification in two classes the best results were obtained with the CART method, that confirmed the importance of phenylalanine as descriptor involved in many diseases.

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## **GEOGRAPHICAL CHARACTERISATION OF HONEYS BY A CHEMOMETRIC APPROACH: A COMPARATIVE STUDY AMONG CLASSIFICATION MODELS**

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Honey contains a mixture of metals and essential elements beneficiary for human health. A multivariate approach was applied for finding a link between geographical origin of honey samples and the measured parameters, being the concentrations of different ions measured by ion chromatography, and parameters that measure the antioxidant activity of the honey samples. The origin of fifty honey samples was divided into three classes, i.e. (a) Eastern European countries, (b) Italy, and (c) Equatorial and other countries. Two different data sets were considered, one with the original parameters and one with normally distributed parameters. Prior to classification, data were divided into a training (35) and a test (15) set using the duplex algorithm (1). Unsupervised methods such as Principal Component Analysis (PCA) (2) and Hierarchical Cluster Analysis (HCA) (3) were applied to visualize the data and to find possible data structures. Then, supervised classification methods like Linear and Quadratic Discriminant Analysis (LDA, QDA) (3) and Classification And Regression Trees (CART) (3) were applied in order to evaluate the existence of data patterns and the relationship between geographical origin and the original or normally distributed parameters, and to classify honey samples according to their origin. The different classification models were compared regarding their predictive ability, simplicity, and interpretability. CART gave the best results, which in addition could be easily interpreted, either applied to the original or to the normally distributed data set. For both LDA and QDA satisfactory results were obtained with five significant variables. In this case the percentages molecules correctly classified from the training set using cross-validation increased passing from autoscaled data set to normally distributed data set, while the % accuracy rate decreased.

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## MIXTURE DESIGN AND CAPILLARY ELECTROPHORESIS AS ANALYTICAL TOOLS FOR THE OPTIMIZATION OF FUNCTIONALIZED NIOSOMES

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Niosomes are vesicular systems formed by non-ionic synthetic surfactants which form double layers and delimitate one or more aqueous components. In this study, N-palmytoyl glucosamine (NPG) functionalized niosomes were developed for brain delivery of the 13 amino acids neuroactive peptide Dynorphin B (Dyn-B). The optimization of NPG-niosomes composition was performed by mixture design, which is a special response surface methodology where the response is function of the different proportions of the components (1). A 13 run-Scheffé Simplex-Centroid Design was employed considering Span 60, Solulan C-24 and cholesterol as mixture components and keeping constant the amounts of NPG, Dyn-B and water volume. The selected responses were the mean vesicle diameter, the polydispersity index (PDI) and drug encapsulation efficacy (EE%). For determining EE%, niosomes were separated from the unencapsulated drug by performing a size exclusion chromatography and vesicles were broken by Triton X-100 addition. Then, the peptide was quantified by capillary electrophoresis (CE), which represents a friendly technique in the field of peptide analysis. In CE problems may arise from the electrostatic interactions between peptides and inner capillary wall, that may generate sensible decrease of efficiency and reproducibility (2). In order to overcome the adsorption, a strongly acidic buffer (pH 2.0 phosphate buffer 50 mM) was used as background electrolyte. In these conditions Dyn-B has a positive electrophoretic mobility due to the presence of three basic amino acids. CE was also used to evaluate the stability of DynB under the same conditions employed for the preparation of niosomes. The ANOVA evidenced that the regression model was significant and valid for the PDI, which decreased by decreasing Solulan C-24. The optimal composition of the niosomes was provided by applying Desirability function (3) and the model predictive capacity was finally demonstrated by comparing calculated and predicted values of the responses.

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## EXPERIMENTAL DESIGN AND CAPILLARY ELECTROPHORESIS IN THE DEVELOPMENT OF CYCLODEXTRIN-BASED HYDROGELS FOR ORAL ADMINISTRATION OF OXAPROZIN

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Oxaprozin (OXA, [3-(4,5-diphenyl-1,3-oxazol-2-yl)propanoic acid]) is a non-steroidal anti-inflammatory drug, which is mainly used for the treatment of various inflammatory disorders such as osteoarthritis and rheumatoid arthritis. OXA belongs to Class II of Biopharmaceutics Classification System (BCS), since it is a highly permeable but very low soluble drug (1). Therefore, an improvement of OXA solubility is particularly useful to increase its oral bioavailability and reduce its dosage, thus reducing the risk of adverse gastrointestinal events. In this study, the possibility of improving the unfavourable chemical-physical properties of OXA by cyclodextrins complexation was investigated. Cyclodextrin (CD)-based hydrogels were developed by using EGDE (Ethylene Glycol Diglycidyl Ether) as cross linking agent, with the aim to control, with an unique mechanism, drug loading and delivery. CD-based hydrogels, made with a fix content in CDs alone or in combination with the polysaccharides hydroxypropylmethylcellulose or dextran at various concentration values, were synthesized according to the method described by Blanco-Fernandez et al. (2). An asymmetric screening matrix was applied to simultaneously evaluate the effect of kind of cyclodextrin, kind and concentration of the other polysaccharides on the mechanical properties and on drug loading capacity of the hydrogels. The drug loading capacity was evaluated by means of a fast short-injection capillary electrophoresis (CE) method. The optimization of the CE method was carried out by applying a Central Composite Design and considering as factors voltage, temperature e concentration of the background electrolyte. The optimized conditions were found by Desirability function (3), and applying these conditions the analysis time was about 0.5 minutes. After having optimized the CD-based hydrogel composition, dissolution rate studies were performed at 37 °C under pH gradient to compare the dissolution profiles of OXA alone or loaded in the CD-hydrogel.

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## DERMAL ABSORPTION OF PLATINUM GROUP ELEMENTS NANOPARTICLES FROM VEHICLES' CATALYSTS

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The platinum group elements (PGEs) represent a new kind of environmental pollutants and a new hazard for human health. Since their introduction as vehicle exhaust catalysts, their emissions into the environment have considerably grown compared with their low natural concentration in the earth crust (1). The PGEs contamination initially occurs in airborne particulate matter, roadside dust, soil, sludge and water, and afterwards results in bioaccumulation in living organisms through different pathways (2). Traditionally, these elements are considered non toxic for human health, but the massive use of Pt, Pd, and Rh as nanoparticles (NPs) and automotive catalytic converters, causing their release in the environment, have determined a new risk factor. In particular, PGE emissions from vehicle catalysts are in the form of nanometer sized particles (3). These elements, both in their metallic form or as ions solubilized in biological media, are now recognized as potent allergens and sensitizers. They have also been associated with asthma, dermatitis and other serious health problems in humans, resulting from chronic exposure at low concentration levels (4). Literature data on the toxicity of these elements in the NPs form and their exposure routes are presently scarce and it's not possible to clearly quantify the risk for human health, in particular that associated with skin exposure.

To fill this gap, a research project on the dermal absorption of PGE NPs was established. The *in vitro* experiments were conducted using 13 nm Pd NPs on intact and damaged human skin using the *in vitro* Franz cell method previously described (5). The amount of Pd determined by ICP-MS in the receiving phase after 24 hours of exposure was  $0.098 \pm 0.067 \mu\text{g cm}^{-2}$  in intact skin and  $1.06 \pm 0.44 \mu\text{g cm}^{-2}$  in damaged skin. Calculated flux and lag time were  $4.5 \text{ ng cm}^{-2} \text{ h}^{-1}$  and 4.0 h for intact skin and  $57 \text{ ng cm}^{-2} \text{ h}^{-1}$  and 4.2 h for damaged skin.

To our knowledge, these are the first data that confirm the permeation of Pd NPs through the human skin and suggest a new potential risk for the health of both workers and general population.

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## STUDY OF THE INTERACTION BETWEEN TREHALOSE CONJUGATED $\beta$ -SHEET BREAKER PEPTIDE AND $A\beta(1-42)$ MONOMERS.

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Amyloid  $\beta$ -peptides are involved in several neuropathological conditions such as Alzheimer's disease (AD). Due to the cytotoxicity of the early intermediate soluble  $A\beta$  species it is important to direct the efforts towards the inhibition of initial steps of amyloid aggregation. Inhibiting  $A\beta$  self-oligomerization could, therefore, provide a useful approach to treating and controlling the pathogenic pathways underlying Alzheimer's disease (AD). Probably, agents that target the molecular recognition process preceding the formation of early intermediates are the most valuable candidates. In our laboratory we have conjugated a trehalose moiety to the known  $\beta$ -sheet breakers pentapeptides LPFFD (1). Trehalose has received special interest because it has been found to be effective in the treatment of neurodegenerative diseases associated with peptide or protein aggregation (2). The glycosidic moiety was covalently linked to the C-terminus of the amino acid sequence. This peptide showed an increased resistance to proteases (1). In this work we investigated the ability of the trehalose conjugated peptide to recognize and bind the monomeric form of  $A\beta(1-42)$ . In this regard, we used several analytical techniques, including Analytical Ultracentrifugation (AUC), Dynamic Light Scattering (DLS) and fluorescence spectroscopy. Furthermore, we have carried out limited proteolysis experiments, which were analysed using ESI-MS, in the attempt of finding out the amino acid region involved in the recognition process.

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## **THE DESTABILIZING ACTIVITY OF FERULIC ACID ON A-BETA PROTOFIBRILS. AN EXPERIMENTAL AND MOLECULAR DYNAMICS SIMULATIONS STUDY.**

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Preventing and reversing the formation of beta-amyloid (Abeta) fibrils under physiological conditions are among the main targets of the therapeutic procedures proposed to date for the treatment of amyloid-related diseases. Current research has indicated that small molecules belonging to different classes of natural compounds could interfere with fibril growth and have even the ability to disassemble preformed folded structures. The small organic molecule ferulic acid (FA), which possesses both hydrophilic and hydrophobic moieties and thus binds peptide/protein molecules through hydrogen bonds and hydrophobic interactions, is a potential candidate against amyloidogenesis. However, the molecular mechanisms connected to this specific action have not been elucidated in detail.

In this study the effects of FA on preformed Abeta fibrils are investigated by means of a concerted experimental-computational approach. Different spectroscopic techniques, such as FTIR, fluorescence, size exclusion chromatography and confocal microscopy in combination with all atom molecular dynamics simulations are used to shed more light on the structural features which play a key role in the destabilization of the aggregates.

Joint experimental and computational studies indicate that FA could have a dual disrupting action on preformed Abeta fibrils in water solution. Indeed, FA redirects the organized conformation of the Abeta fibrils towards amorphous oligomers by means of hydrogen bonding and hydrophobic interactions with the various regions of the oligopeptide assembly. FA molecules interact with each other and form aggregates of various size and shape. This can be correlated with the presence of FA clusters where some of the molecules are bound to the protein surface. These clusters could induce a sort of stabilization and tightening of the protofibril structure in the short term but cause its disruption in the long term, as observed by fluorescence, FTIR, SEC and confocal microscopy experiments.

## **STUDY OF THE INTERACTION OF CHLORINATED AND SULFOCHLORINATED PARAFFINS WITH GELATIN B AND SKIN POWDER. A MODEL FOR FATTENING IN THE LEATHER TANNING PROCESS**

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Fattening is crucial to confer exceptional softness, smoothness and elasticity on leather. Indeed, at the end of the tanning process leather does not contain enough lubricant to prevent it from drying into a hard mass. Fattening agents have the role of lubricating both the surface of collagen fibers and interfibrillar spaces by replacing water.

Chlorinated and sulfo chlorinated paraffins (CPs and SCPs) are common fattening agents employed in the leather industry because it is supposed that they interact effectively with the collagen matrix.

The most abundant collagen type consists of three polypeptide chains arranged in tight triple-helical structures where the conformation of each chain depends on the presence of glycine (GLY) and the high content of proline (PRO) and hydroxyproline (HPR). Fibrillar collagens contain uninterrupted sequences of GLY-X-Y triplets which are flanked by terminal globular domains (telopeptides). A careful analysis of the primary structure of collagen reveals the presence of some patterns and motifs, which may consist of a periodic distribution of certain sequences or features. For example, polar and hydrophobic residues are periodically clustered along the sequence of collagen I every 234 residues.

Differently from fibrillar collagen, gelatin is a heterogeneous mixture of water-soluble proteins of high average molecular masses. These proteins are extracted by boiling skin, tendons, ligaments, bones, etc. in water.

Little is known about the interactions of CPs/SCPs with collagen and gelatin.

In this work we have performed experimental (FTIR spectroscopy) and computational (MD simulations) studies of the interaction of collagen, gelatin and skin powder with CPs and SCPs. The investigation of the reaction mechanisms involved in CPs/SCPs action on collagen can be a good starting point for the development of natural environmental-friendly fattening agents<sup>1</sup> and the definition of more effective and efficient industrial processes.

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## **SIMULTANEOUS DETERMINATION OF LACTATE AND PYRUVATE IN HUMAN SWEAT: A NON INVASIVE APPROACH.**

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Pyruvic and lactic acid are two product of glycolysis. The lactate/pyruvate molar ratio (L/P) in blood is a reliable marker of cell anaerobic metabolism that may occurs in many metabolic and cardiovascular diseases, pulmonary, circulatory and hemoglobin problems. In muscle, during exertion, pyruvate derived from glycogen is reduced to lactate, which is reoxidized and partially reconverted into glycogen during rest. The concentration of blood lactate is usually 1–2 mmol L<sup>-1</sup> at rest, but can rise to over 20 mmol L<sup>-1</sup> during intense exertion because of the switch of muscle cells to a anaerobic metabolism. However, once a certain level of lactate concentration is reached, exhaustion occurs and there is a rapid decline in exercise capacity. Thus, in sport medicine it is used to monitor the maximum performance level of athletes.

The determination of lactate and pyruvate is usually performed in plasma based on the enzymatic reactions. The measurement of metabolites in media other than blood is becoming increasingly significant because of major demands for non-invasive analysis. Such measurements are particularly important to avoid physical and mental strain and infection risk for patients who have to control daily parameters, for people with problems in collecting blood (hemophiliacs, neonates, elderly people), for athletes to estimate their physical and biochemical conditions and to evaluate their training regime.

Here we propose the simultaneous determination of lactate and pyruvate in sweat has been performed using reversed phase high-performance liquid chromatography (RP-HPLC) with UV detection at 220 nm. The calibration curves were linear in the investigated range 0.3 - 350 mM of lactate, 0.003- 1 mM of pyruvate. The sensitivity was good with a limit of detection of 0.03 mM for lactate and 0.001 mM for pyruvate. Recoveries evaluated for the entire procedure were 102 ± 0.1 % and 96 ± 0.1% for lactate and pyruvate, respectively. The method was successfully applied to analysis of sweat in 8 athletes at rest (pilocarpine sweating) and during physical exercise.

Despite the reliability of HPLC measurements, chromatographic approaches are time consuming, require specialized operators and allow only to get off line results. Thus, a further improvement is the development of a novel procedure and device® for the lactate determination “in field”, easy and cheaper with respect to the known methodologies. The method requires an easy and novel modification of common, commercial, portable devices (e.g. devices for the measurement of glucose and lactic acid in blood...) and of their disposable, throwaway reactive electrochemical strips. The method is advantageous both for the low cost of reagents employed and for their short- and long-term stability.

## COMBINED CHITOSAN-SACCHARIDE SCAFFOLDS FOR CONTROLLED PEPTIDE RELEASE

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Chitosan is a  $\beta$ -(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine natural polysaccharide able to form bio- and muco-adhesive hydrogels presenting biocompatibility, low toxicity and biodegradability. Excellent applications of chitosan scaffolds are found in local controlled delivery of both low and high molecular weight drugs. In addition, since chitosan hydrogels present mechanical and compositional similarities with native extracellular matrix they can be successfully used as support for cell growing during tissue regeneration (1).

In this work the attention is focused on the preparation and characterization of chitosan hydrogels with the final goal to obtain scaffolds suitable for tissue regeneration and controlled peptide release. The hydrogels were prepared by pouring a chitosan solution (4.5% in 1% acetic acid) onto a flat surface; then the solution was frozen and subsequently gelled with KOH in ethanol. The distribution, dimensions and number of pores present on the surfaces and into the scaffold were evaluated as a function of six different deposition surfaces (glass, silica, PTFE, cellulose acetate, PVDF and teflon). The viscosity properties of two saccharides (raffinose and saccharose) were tested for they effect on scaffold porosity on each surface. The chitosan scaffolds morphology was then evaluated by scanning electron microscopy (SEM) analysis.

The results obtained showed that the surface microenvironment significantly affected the pore size, homogeneity and distribution.

Since a number of factors (i.e. H-bond, electrostatic bond, pH, drug chemical-physical properties etc.) influences drug-scaffold and drug-scaffold-tissue interactions, the uptake and release of three structurally related compounds (cysteine, cystine and glutathione; an aminoacid, a dipeptide and a tripeptide, respectively) were investigated as a function of pH and ionic strength. Preliminary quantitative results on the uptake and release from chitosan scaffolds of these three compounds investigated were presented by using a purposely developed and validated liquid chromatography-electrospray-mass spectrometry method.

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# LC-ESI-MS DETERMINATION OF HYALURONIC ACID IN DRUG RELEASE EXPERIMENTS FROM PIG INTESTINAL MUCOSA: AN EVALUATION OF MATRIX EFFECT

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Hyaluronic acid (HA) is a non-sulphate natural glycosaminoglycan polymer with variable number of repeating disaccharide units. Presenting extremely wide molecular weight range (from 20 to 4000 kDa) and high hydration level, HA is used in pharmaceutical products for treatment of eye, joint, mucosal and skin fluid-balance disorders.

Drug release experiments on tissue present several analytical challenges, including sample inhomogeneity, presence of endogenous HA, formulation thickness. In this work HA release experiments from two different mucoadhesive polymer-based formulations were carried out. Pig colon mucosa samples were fixed on glass supports and 0.5 mL of HA containing formulation were stratified to obtain almost a 2 mm homogeneous thickness film. Wash-off experiments were carried out to evaluate HA release in phosphate buffer pH 6.8 at 37°C over 24 hours. To evaluate HA concentration in the release medium, a fast liquid chromatography-electrospray-mass spectrometry (LC-ESI-MS) method was developed and validated.

HA containing samples were dried and easily degraded with concentrated sulfuric acid for 10 min at 40°C and then separated on a C18 reversed phase column (100 x 2.0 mm, 3µm). An aqueous acetate buffer/acetonitrile gradient elution was used and positive ion signal was acquired to monitor two selective HA degraded products at m/z 380 and 759 under selected ion monitoring mode, respectively. Since no isotopically labeled standards were available for HA and the sample matrix changed during the wash-off experiment, the ESI matrix effect on HA ionization was evaluated at different times.

The results showed that the method is affected by both absolute and relative (different mucosal parts from the same tissue) matrix effect in terms of systematic proportional error. No systematic constant errors were present. Matrix-matched calibration curves were build at two different times over the whole experimental time to obtain suitable matrix effect correction.

Full validation results demonstrated that this method is useful to reliably and selectively quantify HA kinetic release in the wash-off medium under the conditions used in these experiments.



## ANALYTICAL STRATEGIES FOR PHOSPHOPROTEIN ENRICHMENT FROM CELLS. A CASE STUDY: QUALITATIVE PHOSPHOPROTEOME OF CD4+ T-LYMPHOCYTES FROM NAÏVE HIV-1 INFECTED PATIENTS

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In the last decade, the capability of mass spectrometry to perform comprehensive qualitative and quantitative analysis of cell proteomes and sub-proteomes, providing unique information in the clinical field, was demonstrated in an unbiased fashion manner.

Here, we report the development of a whole analytical procedure for the phosphoproteomic analyses of CD4+ T-cells of naïve HIV-1-infected and healthy donors. The significant analytical challenges related to sample complexity, low number of collectable ex-vivo cells ( $1 \times 10^6$ ) and data number were addressed. We initially focused on the evaluation of different sample treatment protocols for phosphoprotein enrichment. Protein concentration was evaluated for each extract by using aminoacid analysis. Optimal qualitative proteome and phosphoproteome profiles were thus obtained using titanium dioxide tips enrichment method and nano-LC-LTQ-Orbitrap, equipped with collision induced dissociation and multistage activation method. Instrumental parameters were suitably optimized. Proteins were extracted from CD4+ T-cells purified from pooled blood samples of 22 naïve HIV-1-infected individuals or 21 healthy donors. Three independent experiments were performed from each sample. A label-free quantification was performed by adding tryptic BSA peptides as internal standard before desalting and lyophilization process.

Data analysis was carried out by using Proteome Discoverer 1.3 and Mascot algorithm. The analytical approach here presented allowed collection of detailed phosphoproteomic information from very low amount of starting material. Over 100 peptides with different phospho-sites were validated. The results indicate that phosphorylation events targeted proteins with different activity in HIV-1 CD4+ T-cells. In particular, a higher percentage of proteins involved in the catalytic activity, hydrolase activity, ribonucleotide, and ATP binding were revealed in naïve than in donors patients.

These results indicated the applicability of the analytical procedure developed to detect proteins and phosphoproteins from very low amount of samples and the information obtained will be correlated with recent studies based on miRNA signature identification of CD4+T-lymphocytes that discriminate HIV-1-infected from uninfected subjects [1, 2].

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## **XPS CHARACTERIZATION OF CHITOSAN-BASED NANOPARTICLES FOR DRUG-DELIVERY APPLICATIONS**

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Chitosan- and glycol-chitosan thiol conjugates have been developed, in order to realize nanocarriers which can be useful in the transmucosal drug delivery. The aim of the study is to compare the mucoadhesive properties of the two classes of conjugates. Indeed, the presence of thiol groups on the polymer surface is expected both to increase the interaction with the mucin and to promote the absorption of the delivered drugs.

Glutathione and N-acetylcysteine have been chosen to synthesize new thiol-derivatives of glycol chitosan to be compared to the analogous chitosan derivatives (1) in terms of mucoadhesion properties. All the conjugates have been formulated as promising nanoparticles (NPs) for drug delivery.

In the present contribution, x-ray photoelectron spectroscopy has been performed to analyze the surface chemical composition of both the synthesized polymers and the resulting NPs. The preliminary investigations showed the presence of sulphur on the NPs outer shell thus encouraging the eventual surface mucoadhesive properties of the nanocarriers. Further work is in progress to localize the in-depth distribution (2), once the nanocarriers have been loaded with a model drug.

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## SINGLE-WALL CARBON NANOHORNS FOR THE ASSEMBLY OF SCREEN-PRINTED ELECTRODES (SPEs)

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Single-Wall Carbon Nanohorns (SWNHs) were discovered by Iijima (1) and represent a new carbon material having a horn-shaped sheath of single-wall graphitic sheets. They associate each other to form a 'Dahlia- flower'-like aggregate. In this study, SWNHs were characterized by using HR-TEM (High-Resolution Transmission Electron Microscopy), FE-SEM/EDX (Field Emission-Scanning Electron Microscopy), Raman spectroscopy, FT-IR (Fourier Transform-Infrared) spectroscopy, XPS (X-ray Photoelectron Spectroscopy), XRD (X-ray Diffraction) and TG/DTA (Differential Thermogravimetric analysis). Then, a stable and homogeneous SWNHs colloid phase, realized in ethanolic *medium*, was subsequently used to chemically modify SPEs surfaces (2). The modified electrochemical devices were applied for the detection of H<sub>2</sub>O<sub>2</sub>, β-NADH, several neurotransmitters, ascorbic, uric and caffeic acids, guanine and tyrosine, very important targets for interesting bio-medical applications (3).

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## SYNTHESIS AND CHARACTERIZATION OF FUNCTIONALIZED GRAPHENE SHEETS WITH IONIC LIQUIDS (ILS)

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In this study, two different strategies for the synthesis of graphene are reported. In the first case, Oxidized Graphene Nanoribbons (GO) are obtained by the oxidative unzipping of Single-Wall Carbon Nanotubes (SWCNTs), as described in our previous work (1). Then, GO was dispersed in several different ILs and the resulting nano-dispersion were fully characterized, under a morphological and structural point of view (2). In particular, several studies performed by HR-TEM (High Resolution-Transmission Electron Microscopy), FT-IR (Fourier Transform-Infrared spectroscopy) and XPS (X-ray Photoelectron Spectroscopy), demonstrated that ILs were physically adsorbed on the GO surfaces, their edges and walls. The second approach concerns the electrochemical synthesis of graphene gels, covalently functionalized with ILs (3), by the etching of a graphite anode. Also in this case, HR-TEM, FT-IR and XPS analyses revealed interesting information on the interaction between ILs and GO. In particular, nitrogen XP spectra of functionalized GO presented a component ascribable to the coordination of N with electron-rich functionalities. This experimental evidence was observed in all the materials investigated, no matter the IL.

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## BIMETALLIC Pt/Te MICROTUBES MODIFIED ELECTRODE FOR GLUCOSE AMPEROMETRIC DETECTION

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The development of non-enzymatic electrochemical glucose sensors may be a valid alternative among devices employed for glucose detection [1]. The direct electrocatalytic oxidation of glucose on different metal substrates has been reported [2] especially on Pt [3]. A variety of Pt-based bimetallic materials have been fabricated to improve the sensitivity and selectivity of the electrodes towards the electro-oxidation of glucose [4]. Besides, the application of these sensors is slowed down by the toxicities of heavy metal elements, phenomena has often been observed at basic pHs (which are limiting in terms of *in vivo* analysis). As a result, non-enzymatic glucose detection at physiological pH represents one of the goals in sensors technology. Following our previous works on nano/micro-materials and glucose sensors, a bimetallic amperometric sensor for glucose detection based on a Pt electrode modified with Te microtubes (Te-MTs), by direct drop casting of Te-MTs dispersed in ethanol, is proposed. The spectroscopic characterization of as synthesised Te-MTs and Pt/Te-MTs modified electrodes was performed by scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS). Moreover electrochemical characterization of Pt/Te-MTs modified electrodes was performed by cyclic voltammetry (between -600 mV and +800 mV) and chronoamperometry (working potential +200 mV) in phosphate buffer (pH=7 I=0.2). Electrochemical results indicate that the proposed sensor exhibits very strong and sensitive amperometric responses to glucose and explains a good anti-interference ability.

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## NEW METABOLICALLY BIOTINYLATED THERMOSTABLE RED- AND GREEN- EMITTING LUCIFERASES TO IMPROVE MULTIPLEXED BIO-CHEMILUMINESCENT BIOSENSORS

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The availability of probes for ultrasensitive multiplex biosensing is a must to obtain new generation diagnostics able to measure in a single run a wide panel of different disease-specific biomarkers. Thanks to their high detectability, bio-chemiluminescence (BL-CL) based probes are very appropriate for this purpose and they can be easily implemented in miniaturized biosensor devices. Multiplex imaging detection can be achieved by using a sensitive CCD camera and by i) spatially resolving the light emitted by different probes, ii) sequentially measuring the light signals triggered by different substrates (e.g., horseradish peroxidase, HRP, and alkaline phosphatase, AP) and iii) spectrally resolving signals obtained from labels emitting at different wavelengths (e.g., different luciferase mutants).

Two new metabolically synthesized biotinylated *P.pyralis* luciferase mutants were produced and characterized to be used as ultrasensitive universal BL probes. They showed narrow emission bandwidths with 69 nm separation between  $\lambda_{\max}$  (617 and 548 nm) and good thermal- and pH-stability at 25-42°C for up to 6 hours. Probes were captured by streptavidin-coated microtiter plates to compare their analytical performance with that of commercial ones.

The range of linearity for all probes extended at least 5 orders of magnitude over the LOD. Sequential assays of different analytes in the same tube/spot were performed without significant substrate incompatibility between *P.pyralis* and *G.princeps* luciferases, adding AP or HRP substrate at the end of the reading.

Therefore, BL-CL biotinylated probes are suitable for miniaturized multiplexed biosensors. Assisted microfluidics can be used to deliver substrate(s) even in the same spot containing different probes.

## LIGHT INTENSITY BASED OXYGEN OPTICAL SENSOR USING A “SMART” DRIFT CORRECTION ALGORITHM

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A “smart” iterative algorithm able to correct signal drift has been developed. The algorithm may be defined as “smart” because it recognizes the actual condition of the sensing layer by iteratively interpreting the signal of a reference gas (air), allowing the accurate determination of oxygen percentage present in gas mixtures even with the light intensity detection based sensor usually considered unsuitable for that measurement owing to intrinsic weakness of the detection system. Oxidative, photochemical and thermal degradations, acting on both luminophore and polymeric membrane were considered as drift sources. The only requirement for the algorithm correct functioning was the reading of the signal coming from a reference oxygen mixture. Measurements made under severe experimental conditions demonstrated the very good performance of the sensor and ensured even a better performance in mild, more usual, conditions. The algorithm allowed the accurate quantification of the nominal % $O_2$  concentration during nine

days experiment demonstrating to be drift free. In other words it is able to interpret an unpredictable signal drift. On the other hand, the “classical” Stern-Volmer approach, in the same experimental conditions, was useless as it always produced a large positive systematic error increasing with time and with % $O_2$ . Slightly better results were obtained with the correction coming

from the sole sample/reference alternated measurements without the iterative algorithm. In that case a good data smoothing and a significant improvement in the determination of oxygen at concentrations close to the reference gas composition was achieved but correction failed for farther composition. The proposed algorithm gave sensor accuracy as good as the most expensive phase shift based commercial sensors even in the chosen test conditions producing, on purpose, heavy membrane degradation. Even in those drastic conditions, the mean accuracy was  $\Delta\%O_2 = 0.11$ , %RSD

always lower than 5% and close to 1% for higher % $O_2$ .

## **ELECTROCHEMICAL IMMUNOASSAY FOR MUC1 DETECTION AS DIAGNOSTIC TOOLS IN OVARIAN CANCER**

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Considerable efforts have been made in recent years for the development of precise, rapid, sensitive and selective immunosensors for cancer biomarker detection. The aim of the present work is the development of a new screening device for the detection of Mucin 1 (MUC1) protein by using aptamer-modified magnetic beads and graphite based screen-printed electrodes (SPE).

MUC1 is a transmembrane protein, heavily O-glycosylated, found on the apical plasma membrane of most secretory epithelia. In case of a malignant process MUC1 loses its apical distribution, is underglycosylated, overexpressed and is secreted into the blood circulation. MUC1 has been identified as a marker for pre-neoplastic lesions of ovaries, elevated levels of MUC1 protein being involved in tumor progression, especially in the process of metastasis.

Aptamers can be a valid alternative to antibodies or other bio-mimetic receptors, for the development of immunosensors, due to their advantages of ease of manufacture, higher affinity for protein targets, high stability under elevated temperatures and the possibility of discrimination between closely related targets. The assay is based on a sandwich format in which biotinylated aptamers immobilized via streptavidin magnetic beads specifically bind the MUC1 protein. The sandwich assay is performed by adding a secondary and a third alkaline phosphatase (AP)-labeled antibody anti-MUC1. After, the modified magnetic beads are captured by a magnet on the surface of a graphite working electrode and the electrochemical detection is thus achieved through the addition of the AP substrate ( $\alpha$ -naphthyl-phosphate) and  $\alpha$ -naphthol produced during the enzymatic reaction is detected using differential pulse voltammetry (DPV). The conditions for the aptamer immobilization and for the protein binding have been first optimised. The performance of the assay in terms of sensitivity, reproducibility and selectivity has been studied.

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## NEW APTASENSOR FOR VEGF BIOMARKER DETECTION

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The possibility of performing reliable cancer diagnosis even before any symptom of disease appears is crucial for increasing therapeutic treatment success and patient survival rates. During the last decade, improved understanding of carcinogenesis and tumour progression has revealed a large number of potential tumor markers.

Vascular Endothelial Growth Factor (VEGF) is a signaling protein that has been used as biomarker for different diseases, including cancer and when is overexpressed it can contribute to diseases. For this reason rapid and sensitive detection methods must be developed for disease diagnostic and subsequent therapy.

Different detection methods such as enzyme-linked immunosorbent assays (ELISA), immunohistochemistry have been used for VEGF quantification. Nevertheless these methods do not satisfy the rapidity requirement and the necessity to use simple instrumentation for point of care diagnostics. In this context, rapid non-immunochemical sensors based on electrochemical methods that can use aptamers are emerging. Aptamers are single stranded DNA or RNA molecules with a defined three-dimensional shape that allow them to interact with high affinity with a target molecule.

In this work, a new aptasensor for VEGF detection is presented. Different assay formats (sandwich and direct scheme) using gold screen-printed electrodes as transducers have been performed.

Each step of the developed aptasensor assay have been studied and optimized in order to increase the sensitivity and the reproducibility. Differential Pulse Voltammetry Voltammetry and impedance as electrochemical measurements have been used.

Aknowledgments:

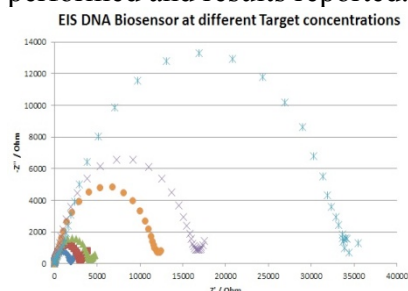
This work has been supported by “Programma azioni integrate Italia – Spagna 2010: Nanobiosensori per la valutazione di marcatori tumorali”.

## ELECTROCHEMICAL BIOSENSING PLATFORMS FOR MICRORNA DETECTION

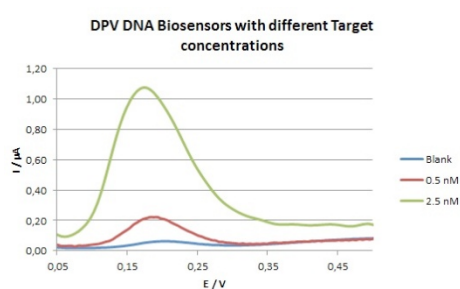
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MicroRNAs (miRNAs) are naturally occurring small RNAs (approximately 22 nucleotides in length) that act as regulators of protein translation. Because many diseases are caused by the misregulated activity of proteins, microRNAs have been implicated 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 research is focused on the determination of microRNAs specific of lung tumors (hsa-mir-221, hsa-mir-222). Electrochemical techniques, such as Faradic Impedance Spectroscopy, Scanning Electrochemistry Microscopy and fast voltammetric techniques (Differential Pulse Voltammetry) was used for the development and characterization of biosensors using Screen Printed Electrodes and enzyme amplification for multiplexing miRNAs detection. The proposed method is based on Thiolated DNA Capture probes immobilized onto gold electrode surfaces (the biosensing platform). Total RNA is extracted from the sample, biotinylated, and then hybridized with the specific capture probes. The biosensing platform was then incubated with streptavidin alkaline phosphatase and exposed to a proper substrate. The product of the enzymatic reaction was electrochemically monitored. The detection of has-miR-222 in Non Small Cell Lung Cancer (NSLC) and Glioblastoma cells performed and results reported.



Impedance spectra (Nyquist plots) shows the influence of target concentration on impedimetric signals.



Differential Pulse Voltammograms shows the influence of target concentration on voltammetric signals.

## ENTRAPMENT OF MOLECULARLY IMPRINTED POLYMER MICROPARTICLES IN ELECTROPOLYMERIZED FILMS FOR ELECTROCHEMICAL SENSING APPLICATIONS

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Among the approaches proposed to prepare electrochemical sensors based on Molecularly Imprinted Polymers (MIPs) (1), MIP electropolymerization (2) allowed polymer synthesis and its integration with the transducer surface in one step. Nevertheless, the best conditions for imprinting and integration steps cannot be separately selected. An interesting alternative, not widely explored so far (3-4), is represented by immobilization of chemically synthesized MIP particles in electrosynthesized films.

In the present work MIP particles are embedded in electropolymerized films and successfully applied in electrochemical sensing of template molecules. The proposed approach allows decoupling MIP synthesis and immobilization, thus enabling the control and the optimization of each step separately. Advantages of electropolymerization (i.e. good adherence of film with controlled thickness to transducer surface) are also kept.

Different chemically-synthesized MIP particles formats (i.e. microspheres and core-shell microsphere by precipitation polymerization, grafted MIP films via iniferter-modified silica beads) have been evaluated using sulfadimethoxine (SDM) as model template. Different conductive polymers (i.e. polypyrrole, poly(3,4-ethylenedioxythiophene)) have been tested as entrapping membranes. The composite materials have been characterized by Scanning Electron Microscopy (SEM) and by Fourier-Transform Infrared (FT-IR) spectroscopy and their performances in SDM electrochemical detection have been evaluated.

The amount of immobilized particles and the thickness of polymer films revealed to play a key role in influencing sensor responses that was higher than that of non-imprinted polymer based sensor. Sensor selectivity was also evaluated by checking the electrochemical response to other sulfonamides.

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## CHARACTERIZATION OF A NEW POLYMERIC PHASE CONTAINING MWCNTs FOR ELECTROANALYTICAL APPLICATIONS

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Carbon Nanotubes (CNT) can be used to prepare smart composite electrodes for analytical applications (1). The preparation of CNT/composites combines the advantages of CNT and bulk composite electrodes.

The fabrication and the characterization of a new carbon nanotube (CNT)/polyethylene (PE) composite electrode, based on the dispersion of CNT within the polymeric matrix in presence of carbon black to improve conductivity, are described. This new CNT/polymeric matrix allows to join high electrical conductivity, chemical stability, mechanical strength, without weakening the electrocatalytic properties of CNT and with an exploitable range of potentials larger than that offered by electrodes chemically modified with CNT.

Various percentages of carbon and MWCNT were tested and, finally, the most performing electrode resulted that containing MWCNT (15%), carbon black (15%) in medium density PE. These percentages are a compromise between analytical performances and mechanical stability of the electrodic material.

MWCNT and carbon black are milled together in an agate mortar before adding PE. The mixture obtained is pressed for 5 minutes at  $3.8 \cdot 10^2$  MPa pressure to obtain a homogeneous tablet, then heated for 8 minutes in an oven at 100° C to soften it for filling the electrode's cavity.

This new electrode has been characterized for its capacitance and by the usual electrochemical investigations with the redox probes  $[\text{Ru}(\text{NH}_3)_6]^{3+/2+}$  and  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ . This electrode was tested for the electroanalytical determination in pharmaceutical formulations of mirtazapine (2), a noradrenergic and serotonergic reuptake inhibitor antidepressant largely used.

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## SELECTIVE AMPEROMETRIC SENSOR FOR TNT BASED ON MOLECULAR IMPRINTED POLYMER AS ELECTROLYTIC MEDIUM

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The need for on-site monitoring of explosives, typically TNT (trinitrotoluene), has been satisfied by different approaches, and in particular by proposing electrochemical probes (1) based on the redox properties of these compounds. Strip cells are particularly convenient for in situ determination because of their low dimension, compactness, low cost and reproducibility. Still interferences can be a problem.

In the present work an amperometric sensor for TNT is investigated, with interesting characteristics of small dimension and good selectivity. It consists of an electrochemical strip cell coated with a layer of an acrylic molecularly imprinted polymer (MIP) specific for TNT which acts at the same time as selective adsorbent and as ionic medium, owing to the presence in the polymer of fixed negative charges, i.e. the carboxylic groups of methacrylic acid, but mobile counter ions.

Two screen printed commercial cells were considered with respectively carbon and gold working electrodes, and one cell obtained by PCB (Printed Circuit Board) etching technique made of gold electroplated on copper. The counter electrode was of the same material as WE, and the reference electrode was Ag/AgCl/KCl sat, or the flat pseudo-reference electrode in the strip cells. The redox properties of TNT at the different working electrodes in aqueous solution were investigated by CV. An irreversible reduction peak at about -0.6V vs Ag/AgCl/KCl sat. was found at all the considered working electrodes at neutral pH, both in solution (1,2) and in MIP as electrolytic medium. The peak currents at bare electrodes were poorly reproducible. In MIP modified cells the peak current was acceptably reproducible at carbon while not at gold electrodes. Surprisingly, amperometry at -0.65 V vs Ag/AgCl gave a reproducible electrochemical response to TNT at both gold and carbon MIP modified cells. The interference from molecular oxygen was high, while the selectivity for aromatic nitroderivatives different from the template molecule, was very good. A similar behaviour was previously found for non electroactive substances (3).

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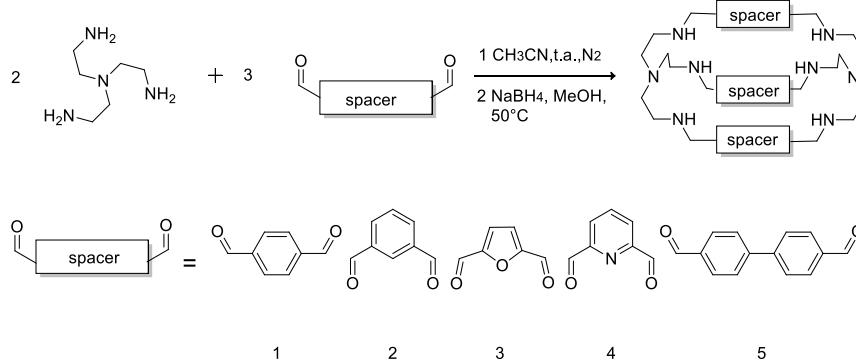
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## RECEPTORS IN SOLID PHASE: DEVELOPING SENSORS FOR ANIONIC RADIONUCLIDES

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The proposed work is a response to the to the cogent need of receptors for the selective binding, separation and extraction of perhenate and pertechnetate ( $\text{TcO}_4^-$  and  $\text{ReO}_4^-$ ). There is a great demand for receptors that would permit the detection, monitoring, selective extraction and separation of pertechnetate from radioactive waste or, more generally, environmental media (1). Unfortunately, the large size and low density of such anions hinder the design of selective receptors; for this reason, receptors able to bind strongly and selectively the two anions are of great interest. Polyamino cryptands, in protonated form, are known to display good affinity for  $\text{ReO}_4^-$  and  $\text{TcO}_4^-$ ; in this work bistren-type systems, in which two tripodal tetra-amine subunits are covalently linked by spacers are considered (Fig.1).



**Figure 1** – Synthesis of the bistren-type ligands for  $\text{ReO}_4^-$  and  $\text{TcO}_4^-$

At this preliminary stage, the nonradioactive  $\text{ReO}_4^-$  was used as a model. Receptors displaying higher selectivity in solution were functionalized and then fixed on polymeric matrixes in order to obtain sorbents that can be employed in the solid phase extraction (SPE) of the considered anions. The first tests, performed as a screening on the performances of the prepared materials, were carried out starting from synthetic solutions at known content of  $\text{ReO}_4^-$ , and evaluating the efficiency of removal, the % of recovery and the selectivity of the polymers. The project is currently underway and in this work we presents some preliminarily results that have made possible the identification of some promising solid phases.

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## SORPTION OF La(III) ON DIFFERENT SOLID PHASES

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The sorption of La(III) on different solid phases is studied by kinetic and thermodynamic experiments. In particular two commercial iminodiacetic sorbents (Chelex 100 resin and Empore<sup>TM</sup> membrane) and two products obtained by functionalization of mesoporous silica with two different ligands, Deferoxamine (DFO) and 1-(3'-Aminopropyl)-3-benzyloxy-2-methyl-4-pyridinone (AcNPr(3,4-HP)) are investigated.

To characterise the sorption, the effects of the solution pH and the contact time are considered. All the experiments are carried out in batch.

Different models are employed to fit the experimental data obtained from both the kinetics and thermodynamic experiments; these models are those regularly applied from our group to characterize different solid phases (1-4). The commercial materials are more effective to sorb La(III) in respect to the functionalized silica.

The sorption of La(III) on Chelex 100 and Empore<sup>TM</sup> involve the formation of the complex  $\overline{LaL}$  between the metal ion and the iminodiacetic groups of the solid phases and the uptake is quantitative at  $pH \geq 3$ . A faster kinetic is obtained with the resin in beads in respect to the membrane; i.e. at pH 5 the equilibrium is reached within 30 min with Chelex 100 and in around 4 hours with Empore<sup>TM</sup>.

The other two materials are mesoporous silica functionalized with DFO and AcNPr(3,4-HP): they are synthesized in our lab and they were initially developed to sorb Fe(III); in the present work we have considered their possible application for La(III).

The sorption for both these solid phases is quantitative only at  $pH \geq 7.5$  and the capacity (mmol La(III)/g dry solid phase) is around 0.2 mmol/g, 10 times lower to the value of the iminodiacetic materials. On the other hand, the rate of sorption is quite rapid reaching equilibrium in around 1 hour.

Although the synthesis of the silica products is less expensive than buying Chelex 100 and Empore<sup>TM</sup>, at the present we can suggest, at least for La(III) sorption, the convenience of use the iminodiacetic commercial materials for applications to real samples.

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## **DEVELOPMENT OF AN ELECTROCHEMICAL AFFINITY BIOSENSOR FOR THE DETECTION OF TUMOR NECROSIS FACTOR ALPHA**

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In this study a commercial human TNF- $\alpha$  matched antibody pairs were used to achieve the analysis of TNF-alpha. The electrochemical bioassay for TNF- $\alpha$  has been developed coupling magnetic beads with screen-printed array of electrodes. At this purpose protein G-coated magnetic beads were modified with the capture antibody. Streptavidin alkaline phosphates conjugate was used as label via biotinylated TNF- $\alpha$  secondary antibody. The modified microparticles were captured by a magnet onto the surface of graphite working electrodes. Electrochemical detection was thus achieved through the addition of enzyme substrate and differential pulse voltammetry was used to measure the product converted at the electrode surface. The use of a disposable screen-printed array of electrodes to perform voltammetric measurements demonstrated that multiplexed electrochemical measurement can be achieved. An increase of the electrochemical signal was obtained upon the increase of the TNF- $\alpha$  in solution and a dose-response curve was obtained. The influence of some parameters such as detection antibody concentration and alkaline phosphatase concentration were evaluated. Moreover, nucleic acid aptamers known to interact with TNF- $\alpha$  selectively, have been tested as detection ligands and results are here reported.



## VOLTAMMETRIC CHARACTERISATION OF SOIL-ISOLATED MICROBIAL COMMUNITY FOR THE DEVELOPMENT OF A MICROBIAL FUEL CELL

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Microbial fuel cells (MFCs) are devices that convert chemical energy to electrical energy through the catalytic activity of microorganisms. In a MFC, electron donors are oxidized at an anode with concomitant production of carbon dioxide, protons, and electrons. The latter are transferred to an anodic electrode. Because of their unique characteristics, MFCs are expected to have a wide range of applications such as wastewater treatment and sustainable energy generation. Hence, research has focused on strategies to enhance the power output of the MFC devices, including exploring more electrochemically active microbes to expand the few already known electricigen families. However, most of the MFC devices are not compatible with high throughput screening for finding microbes with higher electricity generation capabilities. Here, we describe the development of a microfabricated MFC array, a compact and user friendly platform for the identification and characterization of electrochemically active microbes. The MFC array consists of 8 integrated anode and cathode chambers, which function as 8 independent MFCs and support direct and parallel comparisons of microbial electrochemical activities. In this study, we investigated the electrochemical behaviour of microorganisms isolated from soil and in particular of *Enterobacter* EAN3.

## **AUTOMATION OF ULTRATRACE ANALYSIS OF IRON BY ADSORPTIVE STRIPPING VOLTAMMETRY**

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The determination of iron in open oceanic waters is an active research field in chemical oceanography since it was realised that phytoplankton growth in water masses characterised by high nutrient – low chlorophyll (HNLC) levels is limited by the iron content of the water column (1). Its detection is a challenge to analytical chemistry as concentration as low as 0.1 nM (5.6 ng/L) should be detected in a high salinity matrix: moreover, iron is an ubiquitous element, requiring the adoption of strict clean protocols in all of the analytical and preanalytical steps. In this regard, voltammetric techniques have played a major role since decades (see e.g. (2)), although only recently very low levels could be reliably detected (3). At present, voltammetric methods only can directly detect iron without a preconcentration step (see e.g. (4) for a recent ICP-MS procedure with preconcentration).

This presentation will introduce the automation of the voltammetric procedure for the determination of iron by the adsorptive stripping of its complex with dihydroxynaphthalene with catalytic enhancement. Issues related to reagent composition, removal of memory effects and reliable clean procedures will be discussed and the adopted solutions presented. The figures of merit of the procedure were determined, leading to a reliable, automated method for ultratrace analysis of iron in open oceanic waters. The method was validated by analysing round robin oceanic samples.

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## AN ELECTROCHEMICAL METHOD BASED ON SCREEN-PRINTED ELECTRODE COUPLED WITH AN HAEMOLYTIC ASSAY FOR PALYTOXIN DETECTION IN SEAFOOD

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Palytoxin (PITX) is one of the most potent marine toxins known to date. Blooms of *Ostreopsis spp.* have been recently reported along the Mediterranean coasts, causing serious risks to human health. There are neither regulations on PITX-group toxins in shellfish nor official methods for their determination. In order to prevent sanitary risks a provisional limit of 250 µg/kg of shellfish was proposed by the Community Reference Laboratory for Marine Biotoxins. The effect of PITXs is due to their ability to bind the sodium-potassium pump membrane (Na<sup>+</sup>/K<sup>+</sup>-ATPase), causing an ionic imbalance with consequent haemolysis of mammal erythrocytes and alteration of the functioning of excitable cells.

The aim of this work is the development of an electrochemical sensor based on a strip of 8-screen-printed electrodes (8-SPEs) for the detection of PITX and its related compounds. Our method is based on the amperometric measurement of lactic dehydrogenase (*LDH*) released into the medium when sheep erythrocytes are lysed after incubation with PITXs. The degree of haemolysis, and therefore the amount of *LDH* measured, using NADH, pyruvate and appropriate mediators, is correlated to the concentration of these toxins. Two different approaches were investigated. They are based on the use of PMS<sup>+</sup> (phenazine methosulfate) which reacts with NADH producing PMSH. In the first approach PMSH, in the presence of oxygen, gives an equimolar amount of H<sub>2</sub>O<sub>2</sub>. The idea was to measure the hydrogen peroxide with 8-SPEs modified with Prussian Blue. In the second approach PMSH reacts with hexacyanoferrate (III) and the oxidation of the product, hexacyanoferrate (II), is measured at +260 mV. Only the latter approach was proven useful for our purpose. Two different incubation times (24 h and 4 h), between blood sheep and PITX standard solutions were tested, obtaining a working range of 0.007-0.02 ng/ml and 0.15-2 ng/ml, respectively. The specificity of the test for palytoxin was obtained by using ouabain (which prevent the hemolytic action of PITX) and confirmed by the fact that haemolysis was not detected after exposition to high concentrations of other neurotoxins.

Experiments to evaluate the matrix effect on mussel samples, and its possible variability between different samples, are in progress. If this variability will be negligible, a calibration curve in mussel extract could be used for the analysis of experimentally and naturally contaminated samples.

## PRECONCENTRATION AND DETERMINATION OF VANADIUM AS V(V), ON IMMOBILIZED NANOMETER TITANIUM DIOXIDE MICROCOLUMN

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Vanadium is a ubiquitous trace metal in the environment. It exists in different oxidation states, the most common being V(IV) and V(V). This last is the most stable and toxic form of the element (1). In environmental waters V(V) is mainly present as vanadate ion ( $\text{VO}_2^{2+}$  in acidic media and  $\text{VO}_4^{3-}$  in alkaline solution) (2), while V(IV) as vanadyl cation, stable only in acidic medium because of easy oxidation to vanadate by dissolved oxygen at pH higher than 2.4.

In this study, a selective method was developed for the separation of V(IV) and V(V) in water samples by immobilized nanometer  $\text{TiO}_2$  microcolumn and inductively coupled plasma emission spectrometry (ICP-OES). Nanosized titanium dioxide immobilized on silica gel was prepared by sol-gel method starting from a mixture of titanium isopropoxide, isopropanol and water. The coated silica gel was characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM).

V(V) is selectively sorbed on immobilized  $\text{TiO}_2$  at pH around 2.3 while V(IV) remained in solution. Under optimized conditions V(V), retained on the column, was quantitatively eluted with  $\text{NH}_3$  0.1 M. The adsorption capacity for V(V) was found to be  $11.1 \text{ mg g}^{-1}$  also after several separations. Moreover this methods allows the determination of total vanadium in different complex matrices where, after their digestion, vanadium present as V(V), can be preconcentrated on this microcolumn and eluted for its determination. The effectiveness of the optimized procedure has finally been assessed on natural water enriched with the two vanadium species, with satisfactory recoveries ( $> 94\%$ ,  $\text{RSD} < 10\%$ ), and the total determination has been accurately determined in food and reference materials.

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## **TOWARDS THE ESTABLISHMENT OF AN “ENZYME MOLECULAR IMPRINTED POLYMER -SORBENT ASSAY (E-MIP-SA)” FOR MEASURING THE PEPTIDE HORMONE HEPCIDIN.**

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Hepcidin, a peptide hormone produced by the liver, is the main regulator of iron homeostasis (1). Diseases related to an altered iron homeostasis have a high prevalence in the population; moreover, altered iron homeostasis also plays a pathogenic role in several inflammatory and infectious diseases, especially in those with chronic course. Therefore, an accurate measurement of hepcidin levels in biological fluids is potentially of great help in clinical practice. Despite the need of rapid, economic and simple methods for measuring hepcidin, currently, most methods are based on mass spectrometry techniques. The numerous attempts to develop immunoassays for hepcidin have so far yielded limited results because of the structure of hepcidin and its highly level of species conservation make difficult to obtain specific antibodies. Therefore, we approach the development of new (pseudo)immunoassays for hepcidin quantification based on the use of "plastic antibodies". Molecular imprinting technique has attracted considerable attention, because it offers the opportunity of preparing specific antibody-mimic, coupled with several distinct advantages such as excellent stability, ease of preparation and low cost. Although some pseudoimmunoassays exploiting molecularly imprinted polymers (MIP) have been described (2), adaptation of enzyme-linked immunoassays to MIPs has achieved only limited success and so far have not allowed the development of real ELISA-like methods.

With the aim of establishing an ELISA-like assay based on the use of molecular imprinted polymers, MIPs with recognition properties towards hepcidin were directly synthesized into polystyrene wells of microtiter plates. MIPs, covalently attached to polystyrene, played the role of immobilized antibodies. Non-competitive assays with the template, labeled with peroxidase (pp-HRP) were carried out to evaluate binding properties of several "plastic antibodies". The optimal MIP clearly demonstrated selective binding of the pp-HRP. Moreover, competitive experiments carried out in the presence of hepcidin proved that the MIP is also able to recognize the hepcidin molecule. Preliminary results allowed us to conceive the development of an effective "Enzyme MIP-sorbent Assay" (similarly to ELISAs), to effectively quantify hepcidin at levels of clinical relevance.

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## A LATERAL FLOW IMMUNOASSAY FOR THE DETECTION OF OCHRATOXIN A IN WINES

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Ochratoxin A (OTA) is a coumarinic mycotoxin produced by several fungi of *Aspergillus* and *Penicillium* genera. Food contamination due to this toxin occurs in various plant products such as cereals, beans, coffee, dried fruits, and beverages such as beer and wine. The availability of reliable and sensitive analytical methods for the determination of OTA in wine is highly desirable in order to fulfil the need to protect consumers' health from the risk of exposure to the toxin. Thus, rapid, easy and accurate methods are necessary to ensure that the distributed wine products are safe and to allow food quality controls.

In this communication the development of a lateral flow immunoassay (LFIA) for the detection of OTA in wines and grape must is described. The device includes a nitrocellulose membrane on which capturing reagents are immobilized in spatially confined zones and a conjugate pad on which the antibody labelled with gold nanoparticles is pre-adsorbed. Stabilization of gold labelled antibodies was obtained by using ovalbumin. This, combined with the use of PEG in the extraction of OTA from wines, allow us to level the matrix effect caused by the different characteristics of samples (red and white wine) and to control pH. The developed LFIA includes a rapid and very simple treatment of samples that does not involve the use of organic solvents and allows the semi-quantitative determination of OTA at levels as low as  $2 \mu\text{g kg}^{-1}$ . The calibration curve was determined using a non-contaminated white wine fortified with increasing amounts of the toxin. Since the maximum level of OTA in wines is established in  $2 \mu\text{g kg}^{-1}$ , the samples were classified in three categories: positives (above  $3 \mu\text{g kg}^{-1}$ ), negatives (below  $1 \mu\text{g kg}^{-1}$ ) and uncertain (between  $1$  and  $3 \mu\text{g kg}^{-1}$ ). Nine white and twenty two red wine samples were analysed by the developed assay. A good correlation was observed when data were compared with those obtained through a reference HPLC method: 27 on 31 samples were correctly attributed; 2 negative samples were attributed as uncertain and 2 samples contaminated at about  $1 \text{ ng kg}^{-1}$  were incorrectly assigned as negatives. Moreover, the applicability of the assay on grape musts after the addition of 12% ethanol was also verified and results agreeing with HPLC determinations were also obtained on eight grape must samples.

## THE EFFECT OF THE IONIC STRENGTH AND TEMPERATURE ON THE SOLUBILITY AND ACID-BASE PROPERTIES OF DOPAMINE IN NaCl<sub>(aq)</sub>

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Dopamine or 2-(3,4-dihydroxyphenyl)ethylamine is an important neurotransmitter that plays an important role in the regulation of hormonal secretions in the central nervous system and peripheral organs implicated in the control of motor, cognitive and neuroendocrine functions. Its improper regulation is associated with neurological diseases such as parkinsonism, where dopamine levels are reduced, and schizophrenia, which can be related to excess dopamine activity.

It is a precursor of adrenaline and forms in adrenergic nerve endings [tyrosine → 3,4-dihydroxy-phenylalanine → dopamine → noradrenaline → adrenaline]. Owing to the presence of three donor centers (two hydroxy and one amino group) in the molecule, it can form complex compounds with biometal ions. Many biochemical processes occurring in living organisms involve the stages related to a change in the hydration (solvation) state of biomolecules participating in the chemical interaction. The solvation environment plays a considerable role in the complex multistage process of molecular recognition and fixation of physiologically active substances on receptor targets.

In different industrial fields, the knowledge of the drug solubility is a very important property for pharmaceutical product design, because it affects the drug efficacy, its future development and formulation efforts. Since many years, our research group has undertaken a systematic study of the modelling of the acid-base properties and solubility of different ligand classes. The information obtained from this kind of investigation allowed us to determine the total solubility of the ligands, of its neutral species, and the corresponding activity coefficients determined using the Setschenow equation.

The main aim of this paper is to give an important contribution to the knowledge of the solution thermodynamic properties of dopamine, in NaCl<sub>(aq)</sub> at different temperatures and ionic strengths, using the UV-Vis spectrophotometry, spectrofluorimetry and ISE-[H<sup>+</sup>] potentiometry as instrumental techniques. The solubility measurements carried out at different ionic strengths and at T = 298.15 and 310.15 K allowed us to model the dependence of the solubility on the temperature and by means of the Setschenow equation, on the ionic strength.

The dependence of the protonation constants on ionic strength was modelled by means of the Debye-Hückel and SIT (Specific Ion Interaction Theory) approaches, and the specific interaction parameters of the ionic species were determined.

By means of the Van't Hoff equation, the dependence of the protonation constants on the temperature was modelled and the temperature coefficients at different ionic strength values, as well as the protonation entropies were calculated.

## **SPECIATION OF LANTHANIDS(III) IN AQUEOUS SOLUTION: SEQUESTRATION BY REDUCED GLUTATHIONE**

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The number of fields in which the lanthanoid(III) cations ( $\text{Ln}^{3+}$ ) are commonly used is impressive. Their peculiar chemical and physical characteristics make these cations suitable for applications in industries and technologies involving metallurgy, illumination, glass, ceramics, magnets, petroleum, electronics, nuclear energy, and medicine. In this last field, the unique spectroscopic and chemical properties of lanthanoids are exploited for medical imaging and biomedical research purposes. In addition, worth mentioning is the fact that, though no lanthanoids are known to be nutritionally essential in animals or humans, many of these elements can compete with calcium in a number of calcium-mediated biological processes. (2) For all these reasons, it is important to know how lanthanoids behave in biological systems, where their activity, directly dependent on their speciation in aqueous solution, may be influenced by various ligands and other cations. On the basis of the above considerations, our group recently started a systematic study on the speciation of these cations in aqueous solution, with particular reference to natural waters and biological systems (1). This contribution reports the results of an extension of this study to the speciation of various  $\text{Ln}^{3+}$  in the presence of reduced glutathione (or simply glutathione, GSH), in different conditions. The number of key roles played by this peptide in several biological processes makes useless any attempts of summarizing and describing its importance. (3) The effect of temperature, ionic strength and of the kind of lanthanoid on the speciation of GSH /  $\text{Ln}^{3+}$  systems is evaluated, as well as the sequestering ability of this ligand toward these cations. This aspect is analyzed and discussed in terms of calculation of several  $\text{pL}_{50}$  values, an empirical parameter proposed by this group for an objective quantification of the sequestering ability of a ligand in different conditions. (4)

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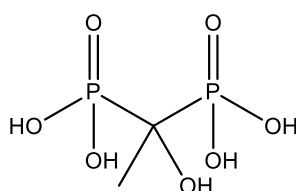
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## ACID-BASE PROPERTIES AND COMPLEXING ABILITY OF ETIDRONIC ACID IN AQUEOUS SOLUTION

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etidronic acid (HEDP)

Etidronic acid (1-hydroxoethane-1,2-diphosphonic acid, HEDP) belongs to a class of chemicals known as phosphonate agents, which are added to water to increase the solubility of certain ions and to inhibit the precipitation of certain mineral compounds. HEDP is a chelating agent that can perform three functions: the sequestration of metal ions that color water supplies or that interfere with the cleaning function of laundry soap or body soap; the action as a scale inhibiting agent that prevents scale formation in commercial heating/cooling systems such as boilers, air conditioners, and cooling towers; prevention of breakdown of oxidizing agents.

In this study, firstly acid-base properties of HEDP at  $t = 25^\circ\text{C}$  in different ionic media and at different ionic strengths ( $\text{NaCl}$ ,  $\text{KCl}$ :  $I \leq 2 \text{ mol L}^{-1}$ ;  $\text{Et}_4\text{NI}$ :  $I \leq 1 \text{ mol L}^{-1}$ ) were studied by potentiometric and calorimetric titrations. The general trend for  $\log K_{\text{H}}$  and  $\Delta H_{\text{H}}$  values is  $(\text{C}_2\text{H}_5)_4\text{NI} \gg \text{KCl} > \text{NaCl}$ .

The dependence of thermodynamic parameters on ionic strength was analysed by an extended Debye-Hückel type equation and the SIT (Specific ion Interaction Theory) approach (1).

Differences in protonation constants in the different supporting electrolytes were also interpreted in terms of weak complex formation by considering the  $\text{M}_i\text{L}$  (with  $i = 1, 2$ ) and  $\text{MLH}_j$  (with  $j = 1, 2, 3$ ) species (with  $\text{L} = \text{HEDP}$ ;  $\text{M} = \text{Na}^+, \text{K}^+$ ).

Moreover, owing the interest in the use of HEDP as water softening agent, formation constants for species formed with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , were determined by potentiometric measurements at  $t = 25^\circ\text{C}$  and  $I = 0.1 \text{ mol L}^{-1}$  in  $\text{NaCl}$ .

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## SEPARATION AND CHARACTERIZATION OF INTACT PROTEINS USING MONOLITHIC CAPILLARY COLUMNS WITH MS DETECTION: EFFECTS OF EXPERIMENTAL PARAMETERS ON SELECTIVITY, EFFICIENCY, MS SPECTRA QUALITY AND OVERALL THROUGHPUT

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New monolithic capillary-nano HPLC columns were prepared by  $\gamma$ -radiation induced polymerization (1) of laurylmethacrylate (LMA) and 1,6-hexanediol dimethacrylate (HDDMA) in presence of a binary porogenic solvent mixture. The poly(LMAcoHDDMA) columns were used for intact protein analysis in Reversed-Phase mode, under gradient elution with UV and ESI-MS detections. A set of 20 different proteins, ranging in MW from 12 to 90 kDA, was used to investigate the effects of experimental parameters on thermodynamic and kinetic performances of the monolithic columns. Selectivity and peak widths (PW) were recorded as a function of varying mobile phase additives (formic, trifluoroacetic and heptafluorobutyric acids, and ammonium acetate at acidic and basic pH), nature of the organic modifiers (acetonitrile, methanol, isopropanol), and column temperature. The use of low levels of trifluoroacetic acid turned out to be the best compromise between chromatographic performances and mass spectrometric detectability. Efficiency of eluted proteins improved significantly upon increasing the column temperature from 40 °C to 60 °C, allowing for short gradient cycle times.

With ESI-MS Orbitrap detection, intense signals arising from multiply charged species were easily obtained. By zooming on selected charge states of medium-sized proteins, we observed that isotopic resolution to baseline is possible for charge states of at least 16. The Xtract and Promass tools gave the intact molecular mass from the signals of the multiply charged protein ions observed in the mass spectrum with isotopic resolution.

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## EVALUATION OF THREE DIFFERENT LOW MOLECULAR WEIGHT PROTEIN PREFRACTIONATION AND ENRICHMENT METHODS FOR PLASMA BIOMARKER DISCOVERY

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Serum low-molecular weight (LMW) proteins contain useful biological information and their identification can be used to discover novel potential biomarkers. When looking for biomarkers in body fluids, blood, including its derivatives plasma and serum, is a logical choice: it can be easily and repeatedly sampled, with minimal invasion, and it can be routinely collected in relatively large quantities. Most importantly, it reflects the physiological and pathological state of an organism. The analysis of proteins as biomarkers in blood, serum or plasma, has become possible with the advent of proteomics technologies and have been employed in several proteomic studies. However, these samples are very difficult to analyze with usual proteomics approaches, due to the very large number of proteins present in the sample and the huge dynamic range of 10-12 orders of magnitude (1), which exceeds the range of concentrations that may be measured by a mass spectrometer (2). Given the high complexity of serum samples, in the last years several different prefractionation and enrichment strategies have been developed. In this work we compared different sample preparation strategies for the study of the LMW proteome of serum, to evaluate which is the best in terms of qualitative and quantitative analysis. In particular, three recent methods suitable for high throughput MS-based proteomics were considered, i.e. hydrogel nanoparticles, Proteominer® peptide ligand affinity beads and Sartorius Vivaspin® molecular weight cutters. For this purpose, we employed a shotgun proteomics approach based on in-solution proteolytic digestion of the whole protein mixture, and determination of the resulting peptides by nanoHPLC coupled with a high-resolution Orbitrap LTQ-XL mass spectrometer. The aim was not to cover entire serum proteome, but to enrich potentially interesting LMW proteins present in whole serum. Data analysis, focusing on the LMW proteome ( $MW \leq 40$  KDa), has shown that the hydrogel nanoparticles performed better in enriching the LMW protein profiles, with 115 proteins identified against 93 and 95 for Proteominer® beads and Sartorius Vivaspin® device, respectively.

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## PAPER SPRAY IONIZATION TANDEM MASS SPECTROMETRY (PS-MS/MS) IN URINARY ACYLCARNITINES ASSAY

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Paper spray (PS) is a recently developed ionization method that has been shown to be effective in analytes determination even in complex biological fluid samples such as whole blood and raw urine. Chromatographic and filter paper are cut into a triangular shape and then loaded with biological samples. When solvent is applied and a high voltage is supplied to the paper, a spray of charged droplets is induced at the tip of the paper triangle (1,2). Paper spray is a fast, direct and low-cost analysis method that has been shown to have some promising features in clinical chemistry (3).

In this work a systematic characterization of paper spray device in the assay of ten urinary acylcarnitines (C<sub>2</sub>-C<sub>18</sub>) was conducted. Urinary acylcarnitines are important biomarkers since the distribution pattern of these species or the excretion of particular acylcarnitines provides some information about metabolic disease (4-6). Early application of desorption ionization methodologies have shown that the underivatized species undergo chemical reaction in the selvedge (7). The performance of different solvent/paper substrate systems were tested using synthetic urine. Papers with various porosity grade were considered along with solvents with different elution efficiency. Furthermore the addition of low polarity solvents was tested. Tandem mass spectrometry in multiple reaction monitoring (MRM) was optimized in order to obtain a better specificity and sensitivity. Analytes signals were evaluated considering its stability and reproducibility.

Internal standards calibration with [8,8,8-d<sub>3</sub>]octanoyl-L-carnitine will be used for accurate quantification. Limit of detection (LOD) and quantification (LOQ) will be determined.

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## URINARY ACYLCARNITINES DETERMINATION BY GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY COUPLED WITH SOLID-PHASE MICROEXTRACTION (SPME-GC-MS/MS)

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Carnitine (3-hydroxy-4-N,N,N-trimethyl-ammonium butyrate) plays a key role in fatty acid oxidation (1). It can be conjugated to fatty acids to form acylcarnitines, which facilitates fatty acid transport into the mitochondrial matrix where oxidation takes place. Acylcarnitines are important biomarkers for various types of diseases including inborn errors of metabolism, and diabetes mellitus type 2 (1-6). Early application of desorption ionization methodologies have shown that the underivatized species undergo chemical reaction in the selvedge (7).

We report a chemical ionization mass spectrometric method for the analysis of ten urinary acylcarnitines (C<sub>2</sub>-C<sub>18</sub>) directly sampled by solid-phase microextraction. Analytes derivatization was carried out using chloroformates and potassium iodide in accordance with the procedure proposed by Sweeley (8). Both chemical ionization (CI) and electron impact ionization (EI) were tested. Mass spectra show the presence of thermal degradation products for longer-chain acylcarnitines. Tandem mass spectrometry in multiple reaction monitoring (MRM) was optimized in order to obtain better specificity and sensitivity. The performances of five SPME fibers and three chloroformates were surveyed taking into account three desorption temperatures for each fiber. The best conditions were used for experimental design optimization by central composite design (CCD) of those variables that affect the derivatization performances (relative proportions of alcohol, chloroformate and pyridine). Sample extraction time and stirring rate, which are variables affecting kinetic aspect, will be optimized by univariate method. Quantification will be realized using [8,8,8-d<sub>3</sub>]octanoyl-L-carnitine as internal standard. Limit of detection (LOD) and quantification (LOQ) will be determined.

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## AN ISOTHERMAL AMPLIFICATION METHOD FOR DNA DETECTION IN DROPLET-BASED MICROFLUIDIC DEVICES

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Nucleic acid amplification is a key method in DNA detection because it generates a great number of target copies, thus increasing assay sensitivity and enabling few-copy nucleic acid detection. The use of microfluidic devices to miniaturize amplification reactions offers significant advantages such as shorter analysis times, faster mass and thermal transfer, reduced sample volume, potential of automation and integration (lab-on-a-chip, micro Total Analysis Systems  $\mu$ TAS). Droplet-based microfluidics, unlike continuous flow systems, creates discrete small volumes using immiscible fluids that are driven into separate microchannels via an independently controlled flow (1). Reagents are confined in droplets and each droplet is isolated from channel walls by the immiscible liquid, greatly reducing sample contaminations and reagent dispersion. Moreover, each droplet can be considered as an isolated microreactor and each microreactor can be individually controlled and analyzed.

The majority of miniaturized systems for nucleic acid analysis are based on the polymerase chain reaction (PCR), which requires thermal cycling between three temperatures during the reaction of amplification of the region of interest of the DNA target. In contrast, low temperature isothermal amplification methods, with no need for thermal cycling, require less energy to operate.

In this communication, the results we obtained in the use of a miniaturized analysis systems using isothermal amplification reactions as alternatives to PCR will be presented. The detection of interaction events in nanoliter droplets have been investigated by using the sensitive DNA detection method based on the isothermal strand displacement polymerization reaction which allows probe/target hybridization, polymerization reaction and target displacement to occur cycle-after cycle, producing, at the same time, an amplified fluorescent signal sufficient to indicate the presence of trace amount of target DNA.

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## SELECTIVE QUANTIFICATION OF NTBI USING IRON SENSITIVE FLUORESCENT BEADS

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Non-transferrin-bound iron (NTBI) occurs in the serum of patients affected by iron overload and it is extremely toxic. Several methods have been reported to quantify this iron pool but the results are not in good agreement (1). None of the present methods are ideal for diagnostic and therapeutic purposes (1). Our intent is to develop a simple, highly sensitive fluorescence-based method which is capable of quantifying NTBI in biological fluids. Different probes have been investigated and we have found that they are able to quantify iron(III) in synthetic solutions. However in the presence of biological fluids (i.e. serum), quantification became impossible due to auto-fluorescence phenomena. The problem was solved using a fluorescent probe covalent bounded to magnetic beads (2). Based on this concept different fluorescent probes (including bidentate and hexadentate pyranones, bidentate and hexadentate pyridinones) have been attached to Dynabeads M-280 and the quenching of the bead fluorescence in the presence of iron overloaded serum samples has been detected. We found that the fluorescent hexadentate pyridinone (3) is the most promising ligand for such quantification NTBI.

The ability of these beads to scavenge iron from Fe-citrate and Fe-albumin complexes will be described and the exchange rate of iron between beads and ligands will also be monitored. In this way we are able to quantify NTBI in the range 0.1 – 20  $\mu$ M using a small sample volume. We also compare our fluorescent-based method with an established method based on the NTA as a chelating agent.

The encouraging results demonstrate the possibility of the development of a standard NTBI quantification assay based on these iron-sensitive fluorescent beads which could be adopted worldwide.

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## DEVELOPMENT OF BRDU-BASED CHEMILUMINESCENT ASSAYS FOR THE QUANTITATIVE DETECTION AND IMAGING OF CELLULAR AND B19 VIRAL LABELLED DNA IN INFECTED CELLS

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Incorporation of exogenous analogues into newly synthesized DNA in living cells is a widely used tool to study DNA replication. Different methodologies based on 5-bromo-2’deoxyuridine (BrdU) *in vivo* labeling are currently available to evaluate cell cycle progression and to disclose potential undesired effects on DNA synthesis upon various cell treatments. BrdU-labelled DNA measurement mainly relies on enzyme linked immunosorbent assays, fluorescence microscopy and flow cytometry. These techniques have been frequently applied in the study of cell proliferation but they are not suited for the simultaneous monitoring of different replicative cycles, as cellular and viral life cycles following infection.

In the present study, chemiluminescence (CL) detection of BrdU was exploited for developing two new methodologies aimed at evaluating BrdU labelling at the single cell level and to measure the amount of cellular and viral DNA produced in the course of an experimental infection. The assays have been optimized on UT7/EpoS1 cells cultured in presence of different concentrations of BrdU and used to monitor parvovirus B19 (B19) life cycle in infected cells.

First, a CL microscope imaging assay has been developed on UT7/EpoS1 cells. The assay allowed objective evaluation of the percentage of replicating cells and the quantitative analysis of BrdU incorporation at single cell level.

In addition, a CL dot-blot assay has been designed to selectively quantify cellular and viral BrdU-labelled DNA, following a B19 capture procedure performed with a specific peptide nucleic acid (PNA) probe. A limit of detection down to  $2 \times 10^6$  B19 genome copies and a linear range extending up to  $5 \times 10^8$  copies were obtained, making the method suitable for monitoring B19 replication in the course of viral infection.

The new methodologies able to discriminate between cellular and viral DNA actively produced in permissive cellular environments from bystander virus in non permissive cells will be a useful tool to define viral interactions with host cells.



## ANALYSIS AT SINGLE CELL LEVEL OF PARVOVIRUS B19 EXPRESSION PROCESS BY CHEMILUMINESCENT MICROSCOPE IMAGING ASSAYS

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Chemiluminescence (CL) microscope imaging represents a powerful tool in life sciences, permitting specific localization and ultrasensitive quantitative detection of the target analyte within cells both in terms of percentage of positive cells and the related CL intensity signal at the single cell level. Herein, CL *in situ* hybridization (ISH) and immunocytochemical (ICH) assays were developed for the quantitative evaluation of parvovirus B19 expression in an experimental infection. Following cellular synchronization, UT7/EpoS1 cells have been infected with B19 and harvested at different hours post infection (in the range 2-72 hpi). At each time point, B19 nucleic acids have been analyzed by three CL-ISH assays enabling the detection of viral DNA, and specific RNAs for non-structural (NS) or capsid (VP1-VP2) proteins. The assays are based on the use of selective digoxigenin-labelled DNA probes detected by horseradish peroxidase (HRP)-conjugate anti-dig antibody and CL detection. Two ICH assays have been performed to detect B19 NS and VP1-VP2 proteins, based on the use of specific antibodies revealed by HRP-conjugate secondary antibodies and CL detection.

The CL imaging methodologies were employed for the monitoring B19 synthesis of nucleic acids and proteins in infected cells. B19 DNA has been detected at constant levels until 24 hpi, then an increasing number of highly positive cells has been imaged with a maximum at 42 hpi (1.3±0.1%) indicating B19 replicative phase. B19 RNAs encoding NS have been detected starting from 2 hpi with the highest number at 24 hpi (0.42±0.03%), before viral replication. On the contrary, B19 RNAs for VP1-VP2 have not been revealed until 12 hpi and the highest number of positive cells has been imaged at 72 hpi (0.38±0.07%), thus following replication. As concerns viral proteins, both non-structural and capsid proteins have been detected starting from 18 hpi, however NS protein detection shows a strictly relationship with RNA detection while VP proteins have been detected later with respect to their RNAs, indicating that capsid protein synthesis is regulated at a post-transcriptional level. Data, being in good agreement with the proposed model for B19 replication in permissive cells, show that the new methodologies, being able to monitor viral replication at the single cell level, represent useful tools to define viral-host interactions.

## INTEGRATED HYDROGENATED AMORPHOUS SILICON PHOTSENSORS FOR CHEMILUMINESCENCE DETECTION IN MICROFLUIDIC BIOANALYTICAL DEVICES

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Chemiluminescence (CL) detection represents a powerful tool bioanalytical miniaturized devices, offering high detectability even in low volumes, high specificity and simple instrumentation required for its measurement, since no photoexcitation source, nor wavelength selection systems are required. Nevertheless, since CL involves the emission of low light levels, highly sensitive detectors are required for its sensitive measurement.

Herein, we reports the characterization of hydrogenated amorphous silicon (a-Si:H) photosensors deposited on glass substrates for the detection of CL reactions in microfluidic devices. The enzyme horseradish peroxidase (HRP), a common label in bioanalysis, was employed as a model and detected by a specific luminol/peroxide/enhancer CL cocktail in a polydimetilsiloxane (PDMS)-glass microfluidic system.

The performance of a-Si:H photosensors, as compared with commercial CCD acquisition system as benchmark, was fully satisfactory for bioanalytical applications, with a limit of detection for HRP down to 100 attomoles and a linear range of response extending up to picomole levels. In addition, spatial resolution and light collection efficiency were characterized.

Preliminary results concerning the application of the developed microfluidic set up for detecting ovalbumin, as a model protein analyte, by a CL non-competitive solid phase immunoassay will be also reported.

The use of integrated a-Si:H photosensors in microfluidic systems paves the way to the development of stand-alone and compact micro total analysis systems ( $\mu$ -TAS) exploiting the features of CL detection, that do not require external and bulky devices for analytical signal detection.

## VALIDATION OF AN HPLC-FLUORESCENCE METHOD FOR THE SIMULTANEOUS FREE AND GLYCOSYLATED PYRIDINIUM CROSSLINKS DETERMINATION IN URINE

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**BACKGROUND:** Pyridinium crosslinks, released during bone resorption, are excreted in urine as free pyridinoline (Pyr) and deoxypyridinoline (D-Pyr), or bound to peptide or to sugars, as galactosyl-pyridinoline (Gal-Pyr) and glucosyl-galactosyl pyridinoline (GluGal-Pyr). Commonly, only total Pyr and D-Pyr urinary amounts (free + bound forms) are evaluated.

**METHOD:** We developed and validated an analytical method based on HPLC-fluorescence for the evaluation of the collagen crosslinks Pyr and D-Pyr (free and total), GluGal-Pyr and Gal-Pyr in the urine of healthy women (n = 20; aged 27-41) and girls (n = 20; aged 5-10). Urine, spiked with an unnatural D-Pyr homologue, as IS, was solid-phase extracted prior to HPLC analysis. The use of this IS and of pure Pyr, D-Pyr, GluGal-Pyr and Gal-Pyr, synthesized to be used as primary calibrators, guarantees the specificity of the method and the correct crosslinks quantification. Total Pyr and D-Pyr amounts were also evaluated after urine hydrolysis.

**RESULTS:** The method demonstrates good selectivity, sensitivity, linearity, precision, accuracy, recovery and stability for all measured crosslinks. Pyr and D-Pyr, both free and total, and GluGal-Pyr amounts were significantly higher in girls than in women ( $p < 0.0001$ ). Gal-Pyr, evaluated in girls for the first time, was under its lower quantification limit ( $< 21.20$  pmol/mL) in women.

**CONCLUSIONS:** The quantification of free and glycosylated pyridinium crosslinks might provide more information on the degradation of various types of collagen, respect to the measurement of total Pyr and D-Pyr alone. Moreover, this validated method could be a useful non-invasive technique for studying pathological conditions characterized by modified glycosylation enzyme activity and for other clinical investigations on bone fragility.

## DETERMINATION OF BOUND AND UNBOUND WARFARIN AND ITS METABOLITES IN HUMAN PLASMA

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Warfarin is the most used anticoagulant for the prevention of thrombosis and thromboembolism. It is a weakly acid drug ( $pK_a = 5.19$ , at  $25\text{ }^\circ\text{C}$ ), highly bound to plasma albumin ( $> 99\%$ ). Only the unbound fraction can carry out a therapeutic action and cross the biological membranes (e.g. salivary glands). The drug is metabolized by the CYP450 system to inactive hydroxylated metabolites (major pathway), and by ketone reductases to warfarin alcohols with a little anticoagulant activity (1, 2).

In this paper, two analytical procedures are presented for the determination of bound and unbound fraction of both warfarin and its metabolites in human plasma. HPLC separation was carried out in isocratic conditions at  $25\text{ }^\circ\text{C}$  on a C-18 reversed-phase column with a 85% buffer phosphate 25 mM at  $\text{pH} = 7$  and 15% acetonitrile mobile phase at a flow rate of 1.2 mL/min. Spectrophotometric detection was performed at 310 nm, and spectrofluorimetric one at 400 nm (excitation wavelength of 310 nm). In both methods, no interference and effect matrix were observed. Analytes recoveries were between 95 and 105% for both total and unbound fractions. The intraday and interday precision were  $<10\%$  (RSD). The methods were successfully applied to pooled plasma samples obtained from patients undergoing warfarin therapy.

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## QUANTIFICATION OF HOMOVANILLIC ACID, VANILLYMANDELIC ACID AND 5-HYDROXYINDOLEACETIC ACID IN HUMAN URINE BY SPME-GC-QqQ-MS AS MARKERS OF NEUROBLASTIC AND CARCINOID TUMORS

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In the presence of neuroblastic and carcinoid tumors, the urinary levels of vanillylmandelic acid (VMA), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) increase due to alteration of the catecholamines metabolism (1,2). Therefore, assay of these acids in urine represents a useful tool for neuroendocrine screening (3). The proposed method is based on sampling of analytes by solid-phase microextraction (SPME) technique and following gas chromatography–triple quadrupole mass spectrometry analysis. Several fibers and three different alkylchloroformates (methyl, ethyl and propyl) were tested to evaluate the affinity between the fibers and derivatized analytes. Best results were obtained derivatizing with ethyl chloroformate and extracting with Polyacrylate fiber in immersion mode. The SPME parameters were optimized by Experimental Design and, in particular a Central Composite Design (CCD) was applied. The optimal values were 25.8 minutes of extraction time, 9.5 %NaCl and 40 °C for extraction temperature. After optimization of the tandem mass spectrometry parameters, the analytical performances were evaluated carrying out calibration curves in the range 0.5 mg/L -100 mg/L, using as internal standards the corresponding deuterated compounds of analytes (HMA-d<sub>5</sub>, VMA-d<sub>3</sub> and 5-HIAA-d<sub>5</sub>). Excellent results were obtained in terms of linearity, accuracy and precision. Limit of detection and limit of quantification values can be also considered satisfactory. The developed method was applied in real urine samples of healthy humans.

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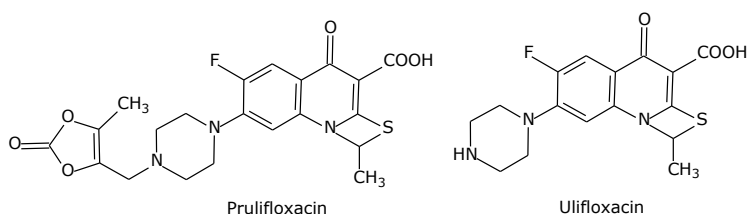
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## HPLC-DAD METHOD FOR THE SIMULTANEOUS DETERMINATION OF PRULIFLOXACIN AND ULIFLOXACIN IN HUMAN PLASMA SAMPLES FOR STABILITY STUDIES

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A new and specific HPLC–UV/Vis method for the direct determination of Prulifloxacin and its active metabolite, Ulifloxacin, in human plasma has been developed. Plasma samples were analysed after a simple SPE clean-up using a new HILIC stationary phase based high-performance liquid chromatography (HPLC) column and an ammonium acetate buffer-acetonitrile mobile phase in isocratic elution mode, with Danofloxacin as the internal standard. Detection was performed using UV/Vis from 200 to 500 nm and quantitative analyses were carried out at 278 nm.



The limit of quantification of the method was 1,0  $\mu\text{g/mL}$  of the cited analytes and the calibration curve showed a good linearity up to 25  $\mu\text{g/mL}$ . For Prulifloxacin the precision (RSD%) and the trueness (bias%) of the method were 13.2% and - 4.94%, respectively. For Ulifloxacin the precision (RSD%) and the trueness (bias%) were 8.30% and 9.03%, respectively. The mean recovery of Prulifloxacin at three concentration levels was 92.2%, while for Ulifloxacin was 103.3%. The method was applied for stability studies in human plasma samples.

## GENDER-SPECIFICITY OF HUMAN BILE ACIDS METABOLOMICS EVALUATED BY HPLC-ES-MS/MS SERUM ANALYSIS

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Bile acids (BA) are the final products of cholesterol catabolism representing its main excretory pathway. BA are signaling molecules interacting with Farnesoid X receptors (FXR) initiating events that reduce the transcription and activity of the rate-limiting enzyme in BA synthesis, cholesterol 7 $\alpha$ -hydroxylase (CYP7A1). BA are also ligands of TGR-5 receptors. These activities are highly BA-structure related. Many BA metabolic pathways are gender related opening more specific and tailored therapeutic intervention. Few data on gender specificity of their serum metabolome are available and in this study an accurate and sensitive HPLC-ES-MS/MS for serum BA analysis has been developed and validated. Separation was achieved on a C18 column and the MS detection was performed by Multiple Reaction Monitoring (MRM) operating in the negative ionization mode. The method is precise (CV=4-12%) and sensitive (LLOQ=0.01 $\mu$ M) and the full metabolome profile of 15 BA was achieved within 30 min. Fasting (8 hours) serum BA was measured in a study population of 135 healthy individuals, 76 male and 59 female, with a mean age respectively of 43  $\pm$ 12 and 40 $\pm$ 13 years. The fasting concentration of total serum BA is significantly higher ( $p=4.8\times 10^{-5}$ ) in men (3.7 $\pm$ 1.8 $\mu$ M) than in women (2.5 $\pm$ 1.3 $\mu$ M). Serum Chenodeoxycholic acid was also significantly higher in men (0.5 $\pm$ 0.5 $\mu$ M) than in women (0.2 $\pm$ 0.2 $\mu$ M) ( $p=2.8\times 10^{-4}$ ). The quali-quantitative composition of other BA is also different. The ratio between Unconjugated and Glycine and Taurine conjugated is significantly higher in the men (0.90) than in the women (0.69). The serum BA metabolome data are in agreement with the BA pool size measured with isotope dilution technique where women present a lower value. Genetic polymorphism in CYP7A1, may explain the gender differences on fasting levels of serum BA. CDCA is highly affected by gender being the more potent CDCA FXR ligand. These results provide evidence for a gender specific BA signaling pathway that may regulate cholesterol homeostasis. The biological and pharmacological significance of these results could be relevant in the design and development of synthetic analogues of FXR agonists and antagonists for clinical applications in cancer prevention, therapy and pharmacogenomics.

## CAPILLARY ELECTROPHORESIS ANALYSIS OF STRESSED *Oenococcus oeni*

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The aim of this work was to study the electrophoretic behaviour of *Oenococcus oeni* (*O. oeni*) under the effect of ethanol stress. The bacterial outer surface of *O. oeni* is rich of ionizable groups (1) so the changes in the charging rates in the optimal and stressed conditions could be evaluated by capillary electrophoresis (2,3).

As a first attempt, it was necessary to optimize the electrophoretic conditions for the identification and efficient separation of this microorganism by capillary electrophoresis. After this preliminary study, the electropherograms show significant differences between cells stressed by ethanol and cells growth in optimal condition. Interestingly, it was observed a substantial difference in electrophoretic profile among different *O. oeni* strains.

The experimental results confirmed the power of capillary electrophoresis for microbial analysis (characterization and separation of microorganisms) since permitting rapid, easy and highly sensitive microbial analysis at low costs for several biological samples.

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## ANALYSIS OF INORGANIC MARKERS OF STRESS IN NATURAL AND GENETICALLY MODIFIED PLANTS IN THE PRESENCE OF CHEMICAL AND PHYSICAL STRESSES.

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The action of biotic and abiotic stresses on plants can induce within the plant the production of compounds able to contrast the effects of the attack. The knowledge of the response of the plants to unfavourable conditions can have useful effects in many fields (biological, environmental, agronomic), taking into account the climatic changes which occur in these last years. (1,2).

The plant that we considered is *Nicotiana langsdorffii* in its wild-type form and transgenic forms for the rat gluco-corticoid receptor gene (GR) and for the rolC gene from *Agrobacterium rhizogenes*. The plant was grown in controlled and reproducible conditions, with the aim of providing a well characterized reference sample and better detect the variations induced by stresses. The investigated plant samples were exposed to chemical (high concentration of chromium) and physical (dehydration) stresses, that give rise to the alteration of the cellular concentrations of a series of inorganic species. We studied such effects, monitoring the modification of a series of ions, such as the concentration of sodium and potassium cations, of nitrate and chloride anions, that are known to be markedly altered both by physical and chemical stress.(3)

The considered cations and some other elements (al, ba, ca, fe, mg, mn, p and si) were determined using icp-oes after acid digestion. Nitrates and chlorides were extracted into water and determined by ion chromatography; the results were compared with those obtained using ion ionoselective electrodes.

The obtained results were treated with multivariate chemometric techniques (principal component analysis and hierarchical cluster analysis) to identify correlations and similarities or dissimilarities among the the different considered markers.

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## **A RAPID HPLC-MS/MS METHOD FOR THE DETERMINATION OF BIOGENIC AMINES IN HUMAN URINE SAMPLES**

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The scientific opinion is increasingly aware of the importance for human health of the so-called biogenic amines. Great importance has been so far devoted to their presence in food (fish, cheese, wines) where they naturally form from aminoacids in the fermentation processes (1,2 and references herein included). Their vasoactive and psychoactive properties make them able to interact with the human metabolism.

More recently the interest also concerns other relevant healthy aspects, since their presence in biological tissues and fluids can represent a marker for different and important diseases, often connected to cancerous effects. The broad group of biogenic amines includes polyamines and catecholamines, whose presence is of particular importance in tissues and biological fluids. Polyamines are involved in cancer cell growth while catecholamines act as neurotransmitters and hormones. Their simultaneous determination is therefore an important task.

In the present work agmatine, cadaverine, dopamine, epinephrine (adrenaline), 2-phenylethylamine, histamine, 3-methoxytyramine, norephedrine, norepinephrine (noradrenaline), octopamine, putrescine, serotonin, spermidine, spermine, tryptamine, and tyramine are simultaneously determined in human urine samples by a HPLC-MS/MS method that does not require a derivatisation step.

To guarantee the maximum of sensitivity, the mass spectrometer works in SRM mode, monitoring for each analyte the two most intense transitions. The method is validated: LODs, LOQs, linearity range, recovery, precision intra and inter day on both concentration and retention time are evaluated. Particular attention is devoted to the matrix effect and the correlated phenomena of ion enhancement or suppression in mass detection.

The method was applied to samples of urine obtained by healthy volunteers of our laboratories. The samples were immediately brought at pH < 2 with trichloroacetic acid to prevent the oxidation of the catechol group, centrifuged, filtered, diluted 1/20 (v/v) in the mobile phase and undergone to HPLC-MS/MS analysis without any other pretreatment.

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## ROLE OF HPLC ANALYSIS ON PALEOGENETIC AND FORENSIC SCIENCES

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Genetic tests based on PCR methodology are widely used for DNA investigation, both in paleopathologic and forensic field. Particular genetic assays called "genotyping" are frequently applied to: (i) the study of genes belonging to HLA system which inform on origins and evolution of pathologies among ancient populations, and (ii) human identification, both for establishing family relationships and for cadaveric recognition. After death, DNA is subjected to hydrolysis of phosphodiester bonds followed by fragmentation. The extent of DNA strand breaks is highly dependent on depurination events and a heavy DNA degradation made it impossible to form genetic assays. Strategies to check the DNA preservation is needed to avoid the failure of expensive and complex genotyping tests. A helpful contribution comes from the determination of D/L-Asp enantiomeric ratio. A comparison between D/L-Asp e D/L-Ala (Alanina) enantiomeric ratio acts as an index of sample "authenticity": in cases where  $D/L\text{ Ala} > D/L\text{ Asp}$ , contaminating DNA is present. For this reason, an RP-HPLC method for D/L determination of Asp and Ala was developed [1]. We performed this RP-HPLC-FL method on bone and blood tissues deriving from human beings belonging to different ages (from 16th century A.C. to modern times) prior to carried out sample genotyping. Data obtained with HPLC are fully in accordance to the results of genotyping assays, where PCR and genetic identification have been successful only for samples characterized by a  $D/L\text{-Asp} < 0.1$  (index of preserved DNA) and D/L-Ala values lower than that of D/L-Asp (index of uncontaminated DNA).

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## **SURFACE PLASMON-ENHANCED FLUORESCENCE SPECTROSCOPY (SPFS) FOR THE DETECTION OF METHYLATED DNA**

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In this work a strategy for the detection of methylated DNA was studied by Surface Plasmon-Enhanced Fluorescence Spectroscopy (SPFS) combined with Surface Plasmon Resonance (SPR).

The strategy is based on the ability of certain protein to discriminate between methylated and non methylated DNA. In this work two protein were used: anti-methylcytosine and Methyl Binding Domain protein (MBD). Both were labeled with an appropriate fluorophore for the SPFS detection. The method studied consisted first in the hybridization of a methylated PCR amplicon (246 mer) on a complementary DNA probe (24 mer) immobilized on the gold sensor surface. Second the protein was flow on the captured PCR amplicon for methylation detection. Suitable experimental conditions and sample pretreatment of PCR samples for the hybridization were studied for the direct detection of the PCR amplicon and for proteins interaction. Different surface functionalization were used: biotinylated probe was anchored to the surface trough streptavidin-biotinylated thiols layers and amino-modified probe was covalently bounded by amino coupling to a thiol layer. Both surfaces were studied in terms of specificity and detection performances.

## STRATEGIES FOR GENOTYPING OF SPECIMENS OF HUMAN ORIGIN BY SPR IMAGING

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We report about SPRi-based sensing applied to SNPs detection on human genomic samples enriched by Whole Genome Amplification (WGA) procedure. In particular, as proof of principle, the rs1045642 SNP was selected on the ABCB1 gene for its potential application to pharmacogenomics. First, a sandwich-like assay was designed, selecting the oligonucleotide sequences by a computational assisted approach. Then the strategy was optimized on a 84 mer synthetic DNA fragment, to accomplish the SNP detection assay to be further used on WGA samples derived from human blood. The direct detection of these type of samples was successfully achieved and the strategy aimed to the discrimination of the polymorphism was confirmed. We demonstrated that this method allows to discriminate between full match and mismatch samples. The biochip resulted well regenerable and reusable for up to 20 measurements.

These promising results can be seen as the starting point for future work oriented toward the direct detection of SNPs in real samples. Moreover, the possibility to perform measurements in multiarray asset by SPRi technique allow to foreseen the possible simultaneous analysis of several SNPs on the same genomic sample. To this aim, different probes specific for a number of genes, could be immobilized on the sensor to screen different SNPs.

## **OPTICAL-BASED SENSING FOR HEPCIDIN DETECTION, A NEW BIOMARKER OF ERYTHROPOIESIS STIMULATORS ABUSE**

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Hepcidin is a cysteine-rich peptide hormone of 25 aa secreted by the liver in response to iron loading and inflammation. Decreased hepcidin leads to tissue iron overload, whereas hepcidin overproduction leads to hypoferremia and the anaemia of inflammation. Hepcidin performs the regulatory activity by binding the iron transporter ferroportin and causing its degradation. Despite the undisputed clinical relevance of hepcidin, its role in human disease has been hampered by the lack of robust and simple assays measuring hepcidin in real matrices. Currently available methods for hepcidin determination have inherent limitations. The poor immunogenicity of hepcidin prevented for years the development of reliable immunoassays. Recently, advances have been made in the development of quantitative methods in biological fluids such as urine and serum, and several reports describing a variety of different assays have been published. These methods are based on immunoassays, or various types of mass spectroscopy (MS)-based protocols, semi-quantitative or quantitative. All of these assays present problems related to quantitative determination and involve expensive equipment and skilled personnel. In this work we present the possibility to face hepcidin detection through the development of affinity-based biosensors, in particular Surface Plasmon Resonance (SPR) and piezoelectric sensing. To this aim, we identified three different bioreceptors specific for hepcidin for a combined approach to its detection. An anti-hepcidin antibody, an anti-hepcidin Spiegelmer (structured mirror-image oligonucleotide), and a 19 aa-synthetic peptide corresponding to the hepcidin-binding site on ferroportin (HBD), have been studied coupled both to SPR and piezoelectric sensing. The performances of the three different receptors have been compared in terms of the main analytical parameters of the relative assays. We believe affinity-based biosensors (ABBs), flanking conventional and profiling methodologies, can contribute to hepcidin detection as a fast, low cost and easy to use instrumental approach.

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## APPLICATION OF GC/MS AND LC/MS-MS TECHNIQUES FOR THE DETERMINATION OF TRAMADOL IN A CASE OF POISONING

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A GC/MS method previously developed and validated in our laboratory (1) has been applied for the systematic toxicological analysis (STA) on blood and urine collected in a case of suspected acute and lethal intoxication caused by tramadol. Tramadol, (1RS, 2RS)-2-[(dimethylamine)methyl]-1-(3-methoxyphenyl)-cyclohexanol, is a centrally acting analgesic drug used for the relief of moderate to chronic pain, showing a weak affinity for the opioid receptors and an inhibition activity on the reuptake of norepinephrine and serotonin (2). In these biological samples bromazepam (deriving from the assumption of Lexotan, an anxiolytic drug) and tramadol have been detected. Due to the particularly high concentration of tramadol found in these samples, which could have caused the death of the person, the distribution of this drug in other biological specimens collected at the autopsy, i. e. bile, gastric content, liver, kidney, lung, brain and hair, has been evaluated

Moreover the biological specimens have been analyzed also by means of the LC/MS-MS technique, which has allowed us to simplify the analytical procedure, thus reducing significantly the time of analysis.

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## ENDOCANNABINOIDS: DETERMINATION IN HUMAN PLASMA BY $\mu$ -SPE FOLLOWED BY HPLC-MS/MS

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Endocannabinoids (ECs) are endogenous compounds that interact with type-1 and type-2 cannabinoid receptors (CB1 and CB2), as well as non-cannabinoid receptors (1). The multitude of roles attributed to ECs makes them an emerging target of pharmacotherapy for a number of disparate diseases (2). Here is presented a high-throughput bioanalytical method based on micro SPE ( $\mu$ -SPE) followed by LC-MS/MS analysis for the simultaneous determination of the two major endocannabinoids 2-arachidonoylglycerol (2-AG) and N-arachidonylethanolamine (anandamide, AEA) in human plasma. The chromatographic conditions, obtained with the fused-core column, allowed a good separation in 10 min also of the AG isomers. A very simple and reliable extraction has been optimized by means of C18 modified tips: it requires only 100  $\mu$ l of plasma and allows the use of minimal volumes of organic solvent (3). A careful optimisation of the extraction step was needed in order to achieve the required sensitivity for the detection of endocannabinoids in plasma. In this respect, extraction recovery, reproducibility and ion suppression from plasma were evaluated as well as capacity. Furthermore loading and elution volumes were studied: they were set at 200  $\mu$ L and 50  $\mu$ L respectively, in order to obtain an enrichment of the analytes of 4 times.

The present method allows a rapid and effective clean-up, which also minimizes the isomerisation of 2-AG. The whole procedure has been validated following the FDA guidelines for bioanalytical methods validation: the satisfactory recovery values, the negligible matrix effect and the good values of accuracy and reproducibility make it a simple and high-throughput analytical tool for clinical and biochemical studies on endocannabinoid signaling in humans.

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## **A RAPID AND SENSITIVE METHOD FOR THE DETERMINATION OF CANNABINOIDS IN ORAL FLUIDS BY MEPS-LC-MS/MS**

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Cannabis is the most widely used illicit drug around the world, so the availability of sensitive and specific analytical methods for its testing in biological fluids is fundamental. The value of oral fluid (OF) as an alternative matrix to document drug exposure is clearly established (1); the advantages of OF also include its non-invasive collection and a reduced risk of adulteration. Saliva is however a complex matrix and a significant issue with its collection is that low sample volumes frequently occurs after cannabis smoking, making difficult direct expectoration. Various collection devices have been introduced to account for this problem, however they could affect the analysis results (2). Currently, the presence of THC is used for the detection of cannabis in OF however only the detection of its metabolite 11-nor-9-carboxy-THC (THC-COOH) provides evidence of active smoking; because of its low concentration, at pg/mL levels, many studies are unable to quantify its concentration.

This study describes a method for the determination of cannabinoids and metabolites in saliva. The sample pre-treatment is based on micro-extraction by packed sorbent (MEPS), a recent technique that uses the basic principles of solid phase extraction, but work with very small volumes of samples (3). Analytes, which include THC, 11-hydroxy-THC, cannabidiol, cannabinol and THC-COOH, are detected by LC-MS/MS. The extraction technique has shown optimal performances on saliva and allowed to work with only 125  $\mu$ L of sample, making possible the collection by simple expectoration; elution volume was set at 50  $\mu$ L in order to obtain an enrichment of the analytes. In these conditions a rapid and effective clean-up has been obtained with satisfactory recovery values and a negligible matrix effect. The chromatographic conditions obtained with fused-core column allowed a good separation of the analytes in only 6 min. The whole procedure has been validated according to SOFT/AAFS guidelines and allows the detection of both cannabinoids and their metabolites in real samples.

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## EXTENSION OF THE POTENTIAL WINDOW ACCESSIBLE TO GOLD NANOELECTRODE ENSEMBLES THROUGH THE DEPOSITION OF BISMUTH FILM

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The use of nanoelectrode ensembles (NEEs) can improve the performance of electroanalytical determinations due to the dramatic increase of the signal to background current ratio with respect to other electrode configurations (1,2). Recently, we showed that the good electroanalytical performance of gold NEEs can be exploited for improving the anodic stripping voltammetric (ASV) determination of very low concentrations of toxic elements, such as arsenic (3). However, at gold NEEs the hydrogen evolution occurs at rather positive potential values, thus restricting the cathodic limit of the accessible potential window (4). This behavior hinders the applicability of gold NEEs for ASV determination of those metal analytes that accumulate and re-oxidize at quite negative potentials.

Recently bismuth was introduced as an efficient replacement for mercury electrodes, also considering its non-toxic character (5). Moreover, the cathodic operational potential window of bismuth film electrode (BiFE) is very similar to that of its mercury counterpart, with superior performance in the presence of dissolved oxygen.

In this work we present a procedure for the deposition of bismuth film on gold NEEs (Bi-NEEs) and its application to the determination of trace levels of lead and nickel ions. Some key operational parameters were optimized using anodic stripping voltammetry for lead, and adsorptive cathodic stripping voltammetry for nickel, reaching detection limits as low as 45 ng/L and 80 ng/L, respectively.

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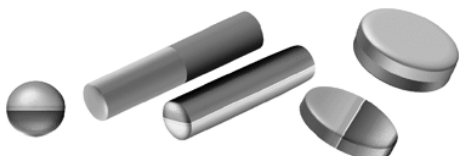
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## PRELIMINARY ASSESSMENT ON THE ELECTROCHEMICAL SYNTHESIS OF 1-D JANUS PARTICLES FOR CHEMICAL SENSING PURPOSES

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The dual nature of Janus particles (JPs) confers upon them fascinating properties. The name of this particles is referred to the Roman god of gates, having two opposite and distinct faces. In practice, the term Janus Particles defines all those particles displaying a dual anisotropic structure, in which two parts with distinct characteristics can be distinguished. Possible architecture for JPs are schematized in Fig. 1.



**Figure 1** Different Janus Particles arrangements.

JPs have been prepared by a variety of methods, including: surface coating, biphasic electrified jetting, photo-polymerization in microfluidic channels, polymer self-assembly (1). Bipolar electrochemistry (2) have been applied to the goal of preparing JPs, with the advantages of requiring simple and cheap instrumentation as well as being potentially suitable for relatively large scale production. In this communication, we describe the electrochemical preparation of Janus like 1-D nanowires (J-NWs) in particular by bipolar electrochemistry and by membrane templated electrochemical deposition (3) exploiting the experimental set-up recently proposed (4). Advantages and limits of bipolar electrochemistry vs. template deposition are critically evaluated, taking into account that the final goal of this study is the synthesis of 1D JPs suitable for analytical applications, based on differentiated self-assembly of the J-NWS in response to changes of the chemical environment in which they are suspended.

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## ELECTROSYNTHESIS OF NANOHYBRID MATERIALS: ELECTROANALYTICAL APPLICATIONS

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Layered Double Hydroxides (LDHs) are lamellar compounds with chemical formula  $[M_a(II)_{1-x}M_b(III)_x(OH)_2]^{x+}(A_{x/n})^{n-} \times mH_2O$ , shortly named  $M_a/M_b$ -A, where  $M_a(II)$  and  $M_b(III)$  are metal cations,  $A^{n-}$  is an anion. Electrodeposition of LDHs based on electrochemical generation of hydroxyl by cathodic reduction of nitrate ions results an efficient method to prepare Ni/Al-LDH thin films, suitable for sensing applications (1,2). In recent years, Au nanoparticles (NPs) have attracted increasing attention due to their unique properties such as high biocompatibility, good conductivity and high catalytic activity (3). Here, we propose a simple one-step electrosynthesis to obtain nanohybrid materials, where Au nanoparticles are entrapped inside the LDH structure, directly on the electrode surface. The proposed procedure displays the advantage of easy fabrication and good repeatability. The resulting AuNPs-LDH composite has been characterized by SEM, TEM, UV-Vis, XRD and electrochemical techniques.

The prepared nanoparticles-LDH composites exhibit high stability and good catalytic activity for methanol electro-oxidation.

We are studying the possibility to extend the use of AuNPs-LDH modified electrodes for the determination of compounds of biomedical interest, such as epinephrine (EP) and dopamine (DA), in the presence of ascorbic and uric acids.

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## CONTROLLED MODIFICATION OF MANUFACTURED GOODS BY COPPER NANO-ANTIMICROBIALS AND THEIR ANALYTICAL AND BIOLOGICAL CHARACTERIZATION

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Colloids of copper nano-particles (CuNPs) to be used as antimicrobials were synthesized by means of an electrolytic process (1) and then used as surface modifiers for industrial batches of manufactured goods such as stuffing, mattresses and textiles. Nanostructured materials, copper-based in particular, have unique physicochemical properties such as ultra small and controllable size, large surface area to mass ratio and high reactivity. Different surface copper loadings can be easily deposited on the surface of the products of interest in order to kill or inhibit the growth of microbes such as bacteria. The chemical speciation of CuNPs was evaluated and correlated to both the electrosynthesis process, storage, and processing conditions. X-ray photoelectron spectroscopy was used to quantitatively assess the materials' surface chemical composition. Optical and transmission electron microscopies were used to morphologically characterize the goods' surface and the CuNPs size, respectively. The antimicrobial properties of Cu-containing products were evaluated on two target microorganisms: gram-positive *Staphylococcus aureus* ATCC 25923 and gram-negative *Escherichia coli* ATCC 25922. Experiments carried out on samples containing very low amount of CuNPs (i.e., surface Cu atomic percentage being as low as 0.5%) produced a strong growth inhibition after only 7 h of contact with both bacteria. Blank and control experiments on goods processed in absence of nano-antimicrobials ensured that the measured biostatic action is due to the CuNPs bioactivity. These promising preliminary results strongly support future real-life applications in different fields.

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## THE USE OF MICROPIPETTE FOR SURFACE PATTERNING. APPLICATION TO ANALYTICAL PURPOSES

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The development of technologies to micro- and nano-patterning surfaces represents an important field of research. The patterning procedures allow the fabrication of micro- and nano-structures that can find applications in various fields, including electronics, chemical transformations and sensing devices. The patterning methods are classified in: bottom-up and top-down [1]. The bottom-up techniques exploit the property of matter to self-assemble and include template synthesis of nanomaterials and self-assembly of block copolymers. The top-down approach allows to achieve higher spatial resolution and comprises photolithography, ion beam lithography and scanning probe microscopy. Among the latter techniques, scanning electrochemical microscopy (SECM) has proven to be a powerful, rather inexpensive tool for patterning purposes [2]. In SECM experiments, the probe is a microelectrode, which is placed close to a substrate, which is immersed in an electrolytic solution. A current response, due to a redox mediator, is recorded at the tip as a function of its  $z$  (approach curve) or  $x$ - $y$  (scanning mode) position above the sample. In this way information on reactivity and topography of the substrate can be acquired. In addition, as lithography, SECM can be used to manipulate the surface and generate micro- and nano-structures. Manipulations include deposition of metals and polymers, functionalization of surface and locally removal part of material to create micropits [3].

In this work, metal patterns have been successfully obtained by coupling SECM and micropipettes, prepared by a laser puller, were filled with the metal salt solution of interest. A pseudo reference electrode was located in the micropipette solution, while the substrate acted as the working electrode [4]. Only the small region wet by a drop of solution, having a diameter about that of the pulled micropipette (10-30  $\mu\text{m}$ ), was involved in the experiments. In this way silver patterns with the desired structures were created, afterwards, each silver spot was functionalized for sensing purposes.

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## ELECTRODEPOSITION OF METALS BY SEBALD

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The Oxygen Reduction Reaction (ORR) is one of the most studied reaction due to its importance in industrial processes, energy systems and corrosion. The ORR takes place in the positive side (cathode) of fuel cells along the interface between a metal and solution through a multistep and multielectron reaction [1]. The high voltage required for the oxygen reduction brings people to use expensive catalysts like Pt. Nowadays fuel cells working in alkaline solution are the best promising devices to introduce new catalyst based on less noble expensive metals that can create a synergic catalytic effect [2]. While some metals like Ag catalyze electronic steps, metals like Fe, Co and Ni promote the adsorption of oxygen on the surface of the catalyst. In order to take advantages from a synergic effect, an electrochemical deposition method controlling the amount of metals is required. Many metals such as Co, Fe and Ni cannot be deposited at underpotential deposition on silver surfaces, and any attempt to control the deposition at overpotential, even at potential slightly negative of the Nernst value did not allow an effective control. The Selective Electrodesorption Based Atomic Layer Deposition (SEBALD) exploits the favorable energy gain involved in the formation of the metal corresponding sulfides in order to control the amount of metals on the surface after a selective electrodesorption of sulfur [3]. We have realized Ag/Fe and Ag/Co/Fe samples by SEBALD using Ag(111) smoothed monocrystal surfaces. A depth study about the electrochemistry of the metals sulfide on Ag surfaces had been required. The samples were tested as catalysts for the ORR in alkaline solution. The interesting catalytic activity shows this expected trend: Ag/Co/Fe > Ag/Fe > Ag. The same trend is found considering the average number of electron exchanged during the oxygen reduction, that show a increasing efficiency and yield of conversion from oxygen to water. SEBALD technique is useful to realize bi-metallic and tri-metallic sample with an un-complete coverage of a metallic surface that could directly concur to the catalytic power of the sample.

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## ELECTROCATALYTIC EFFECT OF ULTRATHIN LAYERS OF SILVER ON GLASSY CARBON

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Oxygen reduction reaction (ORR) and Oxygen Evolution Reaction (OER) play an important role in electrochemical energy conversion systems and in several industrial processes. Bifunctional oxygen/air electrodes are a prerequisite for the development of rechargeable metal/air batteries and/or so called regenerative fuel cells. In fact oxygen is consumed during the battery discharge that produces energy, whereas it is again formed during the battery charge when electrical energy is given to the cell. However, both ORR and OER require high overpotentials that can be lowered only using effective catalysts.

The most effective catalyst is still represented by Pt, and research in the field is increasingly directed to limit the amount of catalyst and to move towards less expensive materials. In our work we tried to follow both goals: in fact we used a less precious metal like silver, whose catalytic properties have been reported long ago [1-5], but also succeeded in limiting its amount by depositing a few  $\mu\text{g cm}^{-2}$  on glassy carbon.

An important result of our experiments was the set up of an activation protocol based on oxidation/reduction cycles that induce the formation and redissolution of oxides of Ag (I) and Ag(II). The catalytic effects towards both ORR and OER increase progressively while increasing the amount of silver. However, the thickest Ag deposit investigated was of about  $16 \mu\text{g cm}^{-2}$ , that corresponds to a theoretical thickness of about 26 nm. This value has been calculated assuming that a monolayer of silver contains about  $1.2 \cdot 10^{15}$  atoms and that its thickness corresponds to the lattice parameter (about 0.41 nm).

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## METHOD VALIDATION OF THE ELEMENTAL CHARACTERIZATION OF NICOTIANA LANGSDORFII BY ICP-LRMS AND ORS-ICP-LRMS

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Characterization of vegetal material for trace elements composition is frequently necessary for its employ. *Nicotiana Langsdorfii* is a variety of plants belonging to genus *Nicotiana* including 62 species and due to its diffusion and variability, it is one of the most studied. Particularly the *Nicotiana Tabacum* variety is used in the smoking material production, reason for the elemental composition knowledge and the detection of toxic elements are indispensable for the consumer's health safe (1).

In this study a method for the sample treatment and elemental analysis of the lyophilized plants of *Nicotiana Langsdorfii* was developed and validated (2). Wild type and genetically modified plants growth in normal or stressed conditions were considered. *Na, Mg, Al, K, Ca, V, Cr, Fe, Co, Ni, Cu, Zn, As, Sr, Cd, Ba, Pb* in mineralized samples were analysed by ICP-QMS. The optimization of the method regarded also the sample treatment. The use of a grinder equipped with PTFE vessels and balls resulted the best solution for sample milling. Different aliquots of the plant samples were compared to define the optimum mass quantity to be digested with ultrapure nitric acid and oxygenated water (3:2 ratio) in a microwave oven. The method repeatability was evaluated by the repetition of 16 sample measurements, while the detection limits of the technique was assessed by blanks. The accuracy assessment and the effectiveness of sample homogenization was investigated by analysis of height aliquots of the certified material NIST 1573a. As and Pb are not certificated, consequently the accuracy for these elements was performed comparing results obtained by ICP-MS analysis with those obtained by hydride generation-atomic fluorescence and anodic stripping voltammetric techniques, respectively. In order to find out the best and simplest analysis conditions for each element, three different analysis mode was compared: ICP-LRMS, ORS-ICP-LRMS using two kinds of collision/reaction gas, He and H<sub>2</sub>. The multitune instrumental method included a total of five analysis mode: the normal mode, without use of any collision/reaction gas and different flows of helium and hydrogen.

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## **USE OF REFUSE DERIVED BIO-ORGANIC SUBSTANCES FOR THE REMEDIATION OF HEAVY METAL CONTAMINATED SOIL**

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The contamination of the ecosystem by metals, often caused by mining and industrial activities, gives rise to serious environmental problems. The remediation of heavy metal contaminated soil is often carried out by soil washing with solutions of chelating agents in order to extract the present pollutants. Chelating agents most commonly used are EDTA, NTA, DTPA or citric acid. These are effective and generally inexpensive but at the end of treatment remain as foreign substances in the treated soil. A more ecofriendly alternative are soluble bio-organic substances (SBO) isolated from biorefuse. On basis of their chemical similarities with natural soil organic matter (SOM), SBO are expected to have no adverse environmental impact on soil. In the present work, the metal complexing and extracting capacity of SBO in aqueous solution has been studied. Six different SBO isolated from different urban biowastes and used in aqueous solution to wash metal polluted soil. The polluted soil-washing solution equilibrium partition time of various investigated metal contaminants versus the soil / washing solution ratio was determined for each of the investigated SBO. Similar data were obtained using washing solutions containing conventional surfactants (sodium dodecyl sulphate) or chelating agents (EDTA, DTPA). The recovered washing solutions, after centrifugation and filtration, were analyzed by ICP for the determination of Cd, Cu, Cr, Ni, Zn and Pb extracted from the soil. Furthermore, the complexation properties of SBO were studied by potentiometric analysis and EPR spectroscopy. The SBO solutions were found as efficient as the above conventional products for removing the investigated metals from the polluted soil.

## COMPARISON OF VARIOUS PHOTOCATALISTS IN THE UV-A AND VISIBLE DEGRADATION OF THE FLUOROQUINOLONE OFLOXACIN

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Fluoroquinolones (FQs) are widespread antibiotics both for human and veterinary use, considered environmental micropollutants (1). Being only partially metabolized in the organism and not completely removed by sewage treatment plants, they are able to reach the environment promoting bacterial resistance. These drugs are persistent against biological degradation (2) but liable to photodegradation and their primary photoproducts were proved to conserve antibiotic activity too.

Ofloxacin (OFL) is one of the most frequently used fluorinated quinolone-type antibiotics, largely detected in river waters because of its long half-life compared to other FQs (1). Heterogeneous TiO<sub>2</sub> photocatalysis is a well established process for degrading a wide range of organic pollutants in water and wastewater, such as FQs (3) and pharmaceuticals in general.

The present work focuses on the evaluation of the effectiveness of different photocatalysts (P25 Degussa, home-made anatase TiO<sub>2</sub>, and home-made nitrogen and sulfur-doped anatase TiO<sub>2</sub>) on OFL removal from natural water at natural pH under actual environmental light conditions (UV-A and visible light). Experiments were carried out under UV-A and solar simulated light. Kinetic constants and photoproducts distribution profiles were compared.

Doping of semiconductor resulted in different degradation rates. First experiments have been conducted at substrate concentration of 10 mg/L in order to evaluate TOC before and after irradiation. Further tests will be performed at ppb levels in environmental waters.

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## OVEROXIDIZED POLYPYRROLE ON GOLD NANOELCTRODE ENSEMBLES: AN IMPEDIMETRIC STUDY FOR DETECTION OF H<sub>2</sub>O<sub>2</sub>

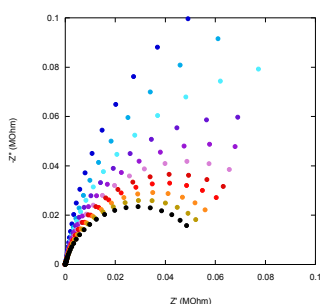
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Overoxidized polypyrrole electrosynthesized on Au nanoelectrode ensembles (NEEs) is here presented as electrode material with suitable properties for sensoristic applications. The gold nanoensembles electrode was prepared following a method known in literature (1) and properly modified to allow the synthesis of polypyrrole.

The polymer grown in an ionic liquid (N-butyl-N-methylpyrrolidiniumbis(trifluoromethanesulfonyl)imide and after overoxidized (2) showed good properties in term of permselectivity toward H<sub>2</sub>O<sub>2</sub> and common biological interferences. The electrochemical impedance spectroscopy (EIS) for H<sub>2</sub>O<sub>2</sub> detection at oPPy/AuNEEs electrode was investigated. The EIS spectra are showed in fig.1. The diameter of semicircles on the real axis represents the charge transfer resistance of the H<sub>2</sub>O<sub>2</sub> reaction on the electrode. The figure clearly shows the wide range of concentration detectable by this electrode material (30-350 mM). An electrochemical characterization of the sensor has been reported.



**Fig.1.** EIS spectra @ overoxidized polypyrrole electrosynthesized on Au nanoelectrode ensembles for H<sub>2</sub>O<sub>2</sub> detection. Concentrations range 30-350 mM in phosphate buffer pH 7.0 in OCV conditions.

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## TeO<sub>2</sub> NANOWIRES AS ADVANCED MATERIALS FOR SENSING: A SPECTROSCOPIC AND ELECTROCHEMICAL INVESTIGATION

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The interest towards Te-based (micro-)nanomaterials is often associated to metal Tellurides or Te(0) in different shapes and sizes. In particular, Te(0) microtubes have been applied in sensing applications for ethanol [1] and glucose [2], just to cite a few. Only recently, some works on TeO<sub>2</sub> nanosystems have also been reported [3] and, among them, the synthesis of TeO<sub>2</sub> nanowires (NWs) by thermal evaporation of Te(0) in an oxygen atmosphere has been described [4]. In view of a sensing application of the material, here we present a direct synthesis of a thick layer of TeO<sub>2</sub> NWs on Pt substrates and a thin layer formation by drop casting of an ethanol dispersion of pre-formed TeO<sub>2</sub> NWs onto Pt surface. Both TeO<sub>2</sub>/Pt systems were characterized by scanning electron microscopy (SEM), X-ray Photoelectron Spectroscopy (XPS) and X-Ray Diffraction (XRD). XPS analysis is especially involved to gain information on the chemical environment of TeO<sub>2</sub> NWs in contact with Pt surface. Additionally, electrochemical characterization of these new modified electrodes TeO<sub>2</sub> NWs/Pt was carried out by Cyclic Voltammetry (CV) and Chronoamperometry (CA) in phosphate buffer (pH=7; I=0.2) to investigate the sensing properties of this material against H<sub>2</sub>O<sub>2</sub>. It was found that tellurium dioxide NWs are sensitive to this analyte.

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## XPS ANALYSIS OF SIZE-SEGREGATED PARTICULATE MATTER FROM A URBAN BACKGROUND SITE IN LECCE

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The application of X-ray Photoelectron Spectroscopy (XPS) to chemical surface analysis of Particulate Matter (PM) is not yet a routine method in aerosol characterisation. Nonetheless, in the last years the interest towards the potentialities of this technique for PM analysis has rapidly grown (1). One of the main advantages of XPS relies on the possible identification, in terms of quantification and chemical speciation, of all the elements (except H and He) present on the particle surface and segregated in ~10-15 nm depth. Surface chemical composition is extremely important to study particulate reactivity. Up to now, only a few works have been reported on the XPS study of size-segregated particles (e.g. PM10, PM2.5 and PM1) (2). In this communication we present an XPS surface study of different size fractions of PM, suitably collected using a 10-stage MOUDI-II impactor (size range 0.056-10  $\mu\text{m}$ ). Results were compared with bulk analysis data of water soluble ions (obtained using High Performance Ion Chromatography) and water soluble carbon (organic and inorganic obtained using catalytic combustion analysis and NDIR detection). Samples were collected onto Al substrates in a urban background site in Lecce and analysed by XPS without any pre-treatment, just cutting a suitable piece (area  $1.0 \times 0.7 \text{cm}^2$ ) of the substrates. Elemental % (conc. > 0.1-1%) surface chemical composition was determined for each size fraction with particular attention to S ( $\text{SO}_4^{2-}$ ),  $\text{Na}^+$ , N ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ), Cl. Detailed analysis of C1s XPS spectra allowed to distinguish oxygen-containing groups such as carbonylic, carboxylic, and carbonate groups. In some cases different hybridisation carbon states  $Csp^2/Csp^3$  associated to different bonds, probably produced during particle formation, were also discriminated (1). Surface and bulk analyses relevant to size fractions characteristic of coarse and accumulation modes are reported, considering also a particular case of Saharan dust intrusion. This study was carried out under the regional project AITECH (Applied Innovation Technologies for Diagnosis and Conservation of Built Heritage).

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## **CHEMICAL CHARACTERIZATION AND SIZE DISTRIBUTION OF AEROSOL IN NY ALESUND (SVALBARD ISLANDS) AND THULE (GREENLAND)**

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Chemical composition and size distribution of atmospheric aerosols give valuable information on sources and processes modifying their properties during atmospheric lifecycle. Such aerosol properties play also a crucial role in the modulating aerosol-radiation interaction and in affecting clouds characteristics. Besides, size distribution influence the long-range transport and the deposition pattern of natural compounds and anthropogenic pollutants. Because of the climatic sensitivity of remote polar areas to the presence of atmospheric aerosols, measurements in these regions are particularly valuable. We report here the results on size distribution and chemical composition of Arctic aerosol sampled at Ny Ålesund (Svalbard Islands, Norway) and Thule (North Greenland) in March - September 2010. Aerosol sampling was carried out using a PM10 sampler (24 h resolution) with Teflon filters. and a 12-stages impactor (SDI, Small Deposit-area Impactor, 4-days resolution) with polycarbonate filters. Analysis was performed by IC, for ionic components, and ICP-SFMS, for selected metals; both techniques are sufficiently sensitive, accurate and reproducible to be applied to very low atmospheric load of aerosol particles, typical of remote polar regions. Moreover, the analysis of the elemental composition of size-segregated SDI samples was carried out by PIXE (Particle Induced X-ray Emission). A detailed aerosol particles size distribution was obtained by a SMPS (Scanning Mobility Particle Sizer) and an APS (Aerodynamic Particle Sizer) devices, able to classify aerosol particles in 106 size classes in the range 10 nm - 20 µm. A large number of channels (54) in the sub-micrometric mode, together with the high temporal resolution (10 min) has allowed the study of the evolution of some events of nucleation and growth of new particles. A comparative analysis of chemical composition, high-resolution particle size-distribution and air masses back-trajectory reconstruction has brought to the identification of specific events of aerosol transport from anthropized continental areas.

## EVIDENCE OF SHIP AEROSOL IN THE CENTRAL MEDITERRANEAN SEA BY THE DETERMINATION OF V AND Ni SOLUBLE AND TOTAL CONTENT BY AES-ICP AND PIXE.

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Ship emissions and their impacts on environment is one of the most important task for atmospheric research, air pollution and climate policy. Studies on ship aerosol contribution are so far based on inventories and modellistic approach<sup>(1)</sup>. The validation of the consistency of ship emissions inventories with observations is a difficult task due to lack of continuous measurements of aerosol chemical composition over the open sea.

We present here the estimate of ship emissions to PM10 in Central Mediterranean Sea by chemical characterization of PM10 sampled at Lampedusa Island (35.5°N, 12.6° E) during years 2004–2008.

Ship emissions are identified using the soluble fraction of V and Ni in HNO<sub>3</sub> at pH1.5 ( $V_{sol}$  and  $Ni_{sol}$ ) determined by AES-ICP. Events of ship aerosol presents  $V_{sol}$  higher than 6 ng m<sup>-3</sup>; this threshold was established on the basis of V/Si enrichment factor with respect to the upper continental crust. The  $V_{sol}$  and  $Ni_{sol}$  are as average 80% of the total V and Ni content determined by PIXE (Particle Induced X-ray Emission) during the identified events of ship aerosol. On the contrary the soluble fraction results less than 40% in events characterized by high crustal content (Saharan dust events).

Air masses back trajectory analysis confirms that the selected events are affected by sea-going ships establishing their origin from the ship tracks crossing the Strait of Sicily. A very intense event in spring 2008 was chemically and size characterised showing that V, Ni but also Al, Fe are distributed in the sub-micrometric fraction of aerosol and they are present as carbonates, idroxides or organic labile complex, so dissolved in HNO<sub>3</sub>.

Data suggest a characteristic  $nssSO_4^{2-}/V$  ratio in the range 200–400 for ship aerosols in summer at Lampedusa. By using the value of 200 a lower limit for the ship aerosol contribution to total sulphates is estimated to account, as a summer average for 1.2 µg m<sup>-3</sup>, representing about 30% of the total  $nssSO_4^{2-}$ , 3.9% of PM10, 8% of PM2.5, and 11% of PM1.

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## CHEMICAL CHARACTERIZATION OF ARCTIC AEROSOL COLLECTED ON OCEANIA SHIP DURING AREX 2011 CRUISE

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The Arctic is a key area, due to its high sensitivity to the climate forcings. Permanent sea ice, ice sheets, snow and permafrost are unique features of the polar regions and amplify the impact of global climate change. In particular, the sea area between Norway and Svalbard Islands (Norwegian and Greenland Seas) is an ideal location to study the combined effects of the climate change affecting the high-latitude Northern-Hemisphere regions.

To improve parameterization and reduce uncertainties in climate models, experimental measurements are necessary to achieve a deeper knowledge on the complex physical and chemical processes that characterize the Arctic troposphere and the air-sea-land interaction.

Size-segregated aerosol was sampled on the top of Oceania ship during the AREX 2011 oceanographic cruise. PM<sub>10</sub> 12-h samples were collected on Teflon filters along several marine transects starting from Tromsø (Norway) to Svalbard Island and along the Western side of Svalbard Islands. Tentative EC-OC samplings (24-h long) were also carried out by a medium-volume sampler with quartz filters.

The chemical characterization of the samples was achieved by different analytical techniques: IC (ion components), ICP-SFMS (selected metals and elements), thermo-optical analyser (EC-OC fractions). In particular, an APEX desolvation system, with an ACM module able to reduce the oxide interferences, was used in order to improve sensitivity and selectivity of ICP-SFMS. In this way, the quantification of ultra-trace (ppt-level) elements, such as Rare Earth Elements (REEs), was achieved.

We report here some preliminary results about the spatial distribution of selected chemical components used as markers of primary and secondary marine aerosol, crustal inputs and long range transport of natural and anthropogenic substances. Back-trajectory re-analysis was used in order to enlighten transport events of continental anthropic emissions. The spatial variability of specific biogenic markers, such as metanesulphonic acid (MSA), will be compared with phytoplanktonic activity (as measured along the water column by a multi-parametric probe) in order to check if a relationship between MSA and marine productivity index, pointed out in the Mediterranean Sea, could be seen also in the Arctic.

## RECONSTRUCTION OF CLIMATIC CONDITIONS IN THE LAST CENTURIES BY THE ICP-AES CHEMICAL ANALYSIS OF ANTARCTIC MARINE SEDIMENT (HOLOCLIP PROJECT)

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One of the main goals of ESF - HOLOCLIP project (Holocene climate variability at high-Southern latitudes: an integrated perspective), is the reconstruction of the Holocene climate and environmental changes from ice and marine cores retrieved in selected areas of Antarctic ocean and ice sheet. In this framework, several marine sediment cores are being analysed for major, minor and trace element content by ICP-AES technique.

Here we present the main results achieved from the chemical analysis of the CB2010 A sediment core, collected in the area of George V Basin (Pacific-Indian sector). The core had been analysed for <sup>210</sup>Pb activities at the EPOC, Université Bordeaux I, and <sup>14</sup>C dating was performed by the Univ. of Trieste, providing independent chronologies and indicating that the core covers roughly the last 300 years.

At the University of Florence, 23 elements were measured (Al, Ba, Be, Ca, Co, Cu, Dy, Eu, Fe, K, La, Mg, Mn, Mo, Na, Ni, Sc, Sr, Ti, V, Y, Yb, Zn), including some REEs and some relevant chemical paleomarkers such as Ba, Mo and Mn. At this purpose, a specific method for sample mineralization was set up, consisting in an acidic (HF+HNO<sub>3</sub>+HCl) digestion in microwave oven, aiming to destroy the silicic as well as the organic matrix. Afterwards, the samples were treated with H<sub>3</sub>BO<sub>3</sub>, in order to neutralize possible remaining HF, whose presence is not recommended for ICP-AES measurements. The method performances were tested on certified marine sediment materials (f.i. MESS-3). Recoveries were always within the certified uncertainty, revealing the method is suitable for this matrix.

The concentration vs. depth record of the measured elements has allowed to enlighten some climate-environment feedbacks. In particular, the trend of the Ba/Al concentration ratio, usually taken as a marker of paleoproductivity, exhibits a good agreement with the pattern shown by the atmospheric temperature at Talos Dome, a site relatively close to the sediment drilling one, as reconstructed by snow  $\delta^{18}\text{O}$  ratio. Such an agreement suggests that a higher atmospheric temperature in the area of George V Land, likely correlated to higher sea surface temperature, could increase the marine productivity. This result looks particularly promising in view of an integration of climatic and environmental records coming from the ice and marine realms.

## RARE EARTH ELEMENTS AND PERSISTENT ORGANIC POLLUTANTS IN THE VENETIAN COASTAL ENVIRONMENT

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The Venetian coastal area is an environment characterized by a strong anthropogenic impact, and its quality level is very important because of local economical activities based on it, like tourism or fishing. In the context of the Water Framework Directive (WFD, 2000/60/EC), the project Q-ALiVe (Qualità dell'Ambiente Litoraneo Veneto) has the aim of taking an image of the quality of the Venetian coastal area, and eventually to find how and where rivers contamination could influence the environmental quality. We studied an area going from the mouth of the Adige river to the Malomocco inlet of the Venice lagoon (including the mouth of the Brenta river and the Chioggia lagoon inlet), to distance from the coast of up to about a kilometer.

In this work we presented the data relative to rare earth elements (REEs) and Persistent Organic Pollutants (POPs) as PCBs, PBDEs and PAHs, in samples of water.

Samples were collected during four different sampling campaigns, covering different seasons (June 2011, august 2011, September 2011, November 2011); in each sampling campaign we collected 10 samples of superficial waters for POPs, 21 superficial waters for trace elements and 21 deep water for trace elements.

Analytical samples procedures for POPs include liquid-liquid continuous extraction, followed by an automated purification step (Power Prep, Fluid Management Systems, USA), with neutral silica columns. Analyse were made by HRGC-HRMS (PCBs) or HRGC-LRMS (PAHs and PBDEs). Quantifications were made by isotope dilution.

The content of REEs, both in non-filtered and filtered water, was determined by sample acidification with HNO<sub>3</sub>, dilution and then analysis by plasma sector field plasma mass spectrometry (ICPSFMS). Quantifications were made by external calibration.

Results suggest a negligible influence of rivers contamination to the quality of the sea facing the city of Chioggia and the Venice lagoon.

Funds for this work were provided, in the framework of Q-ALiVe Project, by the Regione del Veneto - L.R. 15/07.

## TO METHYLATE OR NOT TO METHYLATE? STUDY OF MERCURY SPECIATION ALONG THE VENETIAN LITTORAL SYSTEM (Q-ALIVE PROJECT)

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The biogeochemical cycle of mercury (Hg) is affected not only by the physical, chemical and hydrological characteristics of the environment, but also by changes in productivity and biodiversity. In waters the complexes of Hg are related to the salinity and to the load of dissolved organic carbon (DOC) in the dissolved and in the particulate phases. Surface and bottom seawater were sampled along the Venetian coast at ten sites with different characteristics. Samples were filtered, stored frozen (-20°C) till the analysis in amber bottles, previously cleaned to minimize any contamination. Samples were analyzed according to the method by Cairns et al. (2008), which employs hyphenated techniques (no derivatization steps are required). LOD (limit of detection) and LOQ (limit of quantification) were quantified. Although for some samples both the species were under the LOQ, the presence of CH<sub>3</sub>Hg<sup>+</sup> and Hg<sup>2+</sup> at the same time in surface and in bottom waters were observed. CH<sub>3</sub>Hg<sup>+</sup> concentrations in bottom waters were an order of magnitude higher than those in surface waters. Besides the contribution of sedimentary methylation, that may also be due to the bottom bacterial community, variability in CH<sub>3</sub>Hg<sup>+</sup> concentrations may be due to changes in the phytoplankton communities, which in turn may be affected by nutrient loads from the catchment area and port mouths of the Venice Lagoon. Thus, monitoring these nutrient loads may be essential for the health of the Venetian littoral system, since they may affect blooms, methylation and hyper-bioaccumulation along the trophic web, with effects on the environment and on human health.

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## STUDY OF CHEMICAL POLLUTION IN SEDIMENT CORES FROM THE NADOR LAGOON (MOROCCO). ORGANIC AND INORGANIC CONTAMINANTS

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The north of Africa is currently experiencing a great deal of economic development, which often, as confirmed by many examples, brings about the risk of pollution by toxic chemicals. The Nador Lagoon, the subject of several environmental studies, is located on the northern coast of Morocco. This lagoon is a great point of interest for the natural environment and for the increasing anthropogenic pressure due to adjacent businesses. This work aims to obtain further information about the status of contamination of this lagoon through analysis of sediment cores taken in June 2009. Chemical analyses were carried out of persistent organic compounds (POPs), in particular Polychlorinated biphenyls (PCBs) and Polybrominated diphenyl ethers (PBDEs), as well as the elements of interest for the determination of inorganic contamination. This study addressed four sediment cores taken in some of the most interesting places of the lagoon. For measuring the lagoon sediment chronology the radionuclides <sup>210</sup>Pb and <sup>137</sup>Cs have been used. These also show the possible perturbation of sediments during the years. The sediment dating allowed us to choose the best cores for organic and inorganic chemical analysis. The organic contamination was detected mostly in one sediment core, taking into account the most important samples along the core; for the other three cores only the upper sediment samples have been analyzed. The inorganic contamination was detected in two complete cores. The experimental part concerning the organic compounds involved automatic systems for extraction and purification; the determination was carried out by HRGC-LRMS. The elemental analysis was carried out using analytical protocols already developed in the laboratory: digestion with acids by means of a microwave digester, sample dilution, and then ICP-MS has been used for the analysis. In addition, we used multivariate statistical approaches to develop the results of the analyses and obtain more information about the samples correlation and the origin of the pollution. The Nador lagoon seems to be a not really polluted environment. This analytical research shows the low contamination of the investigated area, moreover shows also the increasing pollution in recent years linked to growing human activity in the surrounding region.

## EFFECT OF IONIC STRENGTH AND MEDIUM COMPOSITION ON THE REMOVAL OF Pb<sup>2+</sup> BY ALGINATE GEL BEADS. DPV-ASV AND ICP-OES MEASUREMENTS

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The biosorption, i.e. a passive sequestering process by metabolically inactive biomass, shows a growing interest for toxic metal ions removal from contaminated aqueous solutions (1), such as municipal and industrial wastewaters. Since the sorption mechanism occurs mainly by ion exchange between the metal ion present in solution and the counter ion of the biopolymer (2), the efficiency of the sorption process largely depends on ionic strength and on the medium composition of solution containing the metal ion to be removed. In order to evaluate quantitatively the influence of ionic strength and medium on the metal removal process by the biomass, we report here results of a study on the sorption capacity of calcium in gel phase toward Pb<sup>2+</sup> in aqueous solution in different simple (NaCl, NaNO<sub>3</sub>) and mixed [NaCl+CaCl<sub>2</sub>, NaCl+MgCl<sub>2</sub>, NaCl+Na<sub>2</sub>SO<sub>4</sub>) ionic media, in a wide range of ionic strength ( $0.05 < I/\text{mol L}^{-1} < 0.8$ ), and at room temperature. Investigations were performed by evaluating the Pb<sup>2+</sup> concentration in the solution after adsorption onto different amounts of calcium alginate gel beads over continuous time. Measurements of Pb<sup>2+</sup> concentration were carried out by Differential Pulse Anodic Stripping Voltammetry (DP-ASV) and by Inductively Coupled Plasma Optical Emission Spectroscopy ICP - OES. To avoid the hydrolysis of the of Pb<sup>2+</sup> ion, the solution containing the metal ion to be removed was kept at pH ~ 5. The pseudo second-order model was used to fit sorption process kinetic data (3). Sorption equilibrium was analysed using Langmuir and Freundlich isotherm models. Although both isotherm equations fitted properly the equilibrium data, Langmuir model seems to fit data slightly better than the Freundlich model.

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## SORPTION OF $Cd^{2+}$ AND $Cu^{2+}$ IONS FROM AQUEOUS SOLUTIONS BY ALGINATE AND ALGINATE/PECTIN GEL BEADS

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Removal of toxic metal ions from natural waters, wastewaters and contaminated sites is of great importance for the health of living organism and for environmental protection (1). Alternatively to the conventional chemical treatments, such as precipitation, reverse osmosis, etc, that are expensive and of high environmental impact, the use of low cost and environmental friendly sorbent materials is a very promising new technology for metal ions removal (2,3). The most investigated sorbent materials are of organic origin derived from the natural biomass (algae, fungi, bacteria) and from industrial processes, such as wood, agriculture, fishery, textile manufacturing, etc. Also some inorganic materials (zeolites, clays, etc.) are used for the same purpose (4). All these sorbent materials show characteristics of low cost, low environmental impact, high degradability, great availability and high sorption capacity towards metal ions. The sorption ability of these materials is strictly related to their chemical composition containing high percentages of macromolecules with a great number of functional binding groups (-O, -N, and -S donors). Here we report results from an investigation carried out to remove cadmium(II) and copper(II) from aqueous solutions using alginate and pectin in gel phase. Both these materials are able to form hydrogels in the presence of small amounts of divalent cations, especially calcium ion. In this study we report equilibrium and kinetic investigations on biosorption of Cd(II) and Cu(II) from aqueous solutions by alginate and alginate/pectin gel beads. Batch kinetic measurements performed by varying the type of metal and the composition of the spherules at pH value near to 5 in order to avoid the hydrolysis processes of Cd(II) and Cu(II) showed that the sorption follows a pseudo second-order kinetic model and the sorption rate increases with pectin concentration in the gel beads. The equilibrium data were fitted by using both Langmuir and Freundlich isotherm models and a comparison was made among results obtained.

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## **TRACE ELEMENTS, PAHs, PBDEs AND PCNs ON ULTRAFINE PARTICLES IN THE URBAN AREA OF VENICE.**

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Nanoparticles emissions are associated with impacts on cardiopulmonary health as well as on increase in mortality. The emission, evolution and exposure-uptake of particles with aerodynamic diameter  $\leq 100$  nm are fundamentally quantified by their number concentration (number of particles/m<sup>3</sup>) as a function of particle size. Nanoparticles number distributions are widely variable and change fast, as their concentrations are strongly influenced by local environmental conditions.

Ultrafine particles were collected on the roof of the Department of Environmental, Informatics and Statistics Sciences of the Ca'Foscari University of Venice, using a low-pressure impactor (DLPI DEKATI). The impactor classifies 13 size fractions, from 0.03 to 10  $\mu\text{m}$ .

The better interval time has been established for obtain the best definition for the chemical species detected. As a matter of fact, few hours of sampling did not give an optimal detection for chemical analysis. Based on the elapsed time for sampling and on the best analytical detection, in this work are reported samples, collected in August and in December 2010. The organic compounds (PAHs, PBDEs and PCNs) and elements contents have been analyzed for each fractions in all samples. The results show that organic contents were higher in the December samples highlighting a different size distribution.

The distributions of elements show a concentration decreasing in the class 1 $\mu\text{m}$  where the most abundant elements were Na and Br.



## **PAHs IN SARAJEVO CITY: GAS PHASE DISTRIBUTION IN NIGHT AND DAY SAMPLES, SOURCE RECOGNITION AND HUMAN INHALATION RISK**

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Polycyclic Aromatic Hydrocarbons (PAHs) are organic pollutants derived from pyrolysis and pyrosynthesis processes; as well as through spillage of oil during transport –petrogenic-. Industrial activity, motor vehicle emission, domestic combustion are the main source of PAHs in urban atmosphere. They are released into the environment via the atmosphere often associated with soot or black carbon.

Little information is found in the literature about the levels of PAHs in urban air distinguishing day and night time. In this work we want to separate the PAHs collected during the day and the night-time, and through we applied the diagnostic ratio to identify the possible sources of PAHs; and finally established the risk level using a risk index in Sarajevo city.

The result of this study suggest that: (i) the total PAHs (gaseous + particulate phase) concentrations values were similar to those reported in other European cities; (ii) the PAHs daytime concentrations are higher than night-time (iii) combustion and traffic were main sources of PAHs; (iv) the B(a)P concentration in particulate phase is higher than EU limit value - average annual value - ( $1 \text{ ng/m}^3$ ); v) PAHs cancer risk exceeds the carcinogenic benchmark level recommended by the EPA.

## SEC-ICPMS TO STUDY THE INTERACTION BETWEEN METALS AND HUMIC SUBSTANCES

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Dissolved organic matter (DOM) covers an important role in the environment. It influences the transport and the distribution of organic and inorganic components through different processes such as complexation and precipitation of elements or adsorption of organic contaminants (1). Dissolved humic substances (HS), that represent the greater part of DOM in environment samples, are amorphous, acidic and polydisperse, with molecular weights (MW) ranging from low value (1kDa) to much larger entities. They are constituted from 2 main fractions that are humic acid (HA), soluble in base and fulvic acids (FA) soluble in both acid and base.

To define adequate separation methods is particularly important to examine the HS interactions with metals (2). Some studies suggest that the capacity in complexing metal ions changes with MW and charge density, as well the cation (3).

The aim of the present study was the dimensional characterization of HA extract from different matrix and geographic areas, analyzing also the metal distribution among the different dimensional classes and the complexing capacity. High performance size-exclusion chromatography was chosen as separation technique, equipped with an UV-vis absorption spectrometry detector and ICP-MS for multi-element-specific analysis. The eluent composition, pH and ionic strength was investigated in order to find out the best separation condition. Polystyrene Sulfonates (PSS) were used for the calibration of the polymeric TSK-GEL G3000PW<sub>XL</sub> column. The wavelength 270 and 335 resulted the most appropriate, when UV detection was used. The results showed that the best eluent was a solution of THAM 10 mM and Ammonium phosphate 2.5mM, pH 8.

The content of all the HA studied was determined by the ICP-MS analysis. A different distribution of the metals among the different dimensional fractions of the organic substances was indicated on the base of the retention times.

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## DEVELOPMENT OF A SPME/GC/MS METHOD FOR THE SIMULTANEOUS DETERMINATION OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN ENVIRONMENTAL WATERS

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Pharmaceuticals and personal care products (ppcps) constitute a broad class of emerging contaminants, belonging to the list of high production volume chemicals that are currently used for human and veterinary application (e.g., pharmaceuticals, sunscreens, cosmetics, insect repellents, and soaps) (1,2). These substances have been continuously discharged to the aquatic environment, with resulting environmental and human health impact.

In this work, a headspace solid phase microextraction (HS-SPME) method coupled with gas chromatography and MS detection (GC/MS) was optimized for the simultaneous determination of 21 target PPCPs in water samples. The analytes included fragrances, UV-filters, antiseptics, estrogens, anti-inflammatory drugs and pesticides. SPME is an environmentally-friendly alternative to the common approaches, because it achieves extraction and clean-up in a single step eliminating the need for solvents and expensive equipments (3). An on-fiber SPME derivatization, using silyl reagents, was performed for the analysis of more polar acidic compounds. An experimental design approach was applied to systematically investigate and optimize the operative parameters affecting the extraction recovery: extraction temperature and time, derivatization time, desorption temperature and time. The optimal conditions were: extraction time of 125 min at 40°C; derivatization time of 30.5 min; desorption time of 2 min at 300°C. Under these conditions, good repeatability was assessed as RDS% values  $\leq 10\%$  for underivatized PPCPs and  $\leq 20\%$  for derivatized ones. The method detection limits were between 0.7 and 9.0 ng L<sup>-1</sup>, with the highest values in the range 2.5-9.0 ng L<sup>-1</sup> for the derivatized analytes. Method accuracy was evaluated on spiked tap water samples: recoveries varied from 85 to 103% and from 75 to 110 % for non derivatized and derivatized compounds, respectively. The results obtained indicate the potentiality of the method for the determination of the investigated analytes at trace levels.

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## GC-MS METHOD FOR SIMULTANEOUS ANALYSIS OF DICARBOXYLIC ACIDS AND SUGARS IN ATMOSPHERIC AEROSOL: RESPONSE SURFACE METHODOLOGY FOR OPTIMIZING SOLVENT EXTRACTION

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Water-soluble organic compounds (WSOCs) are an important group of chemical tracers that may give relevant information on the relative strengths of primary emission sources and secondary photochemical processes on air quality. In fact, they can be primarily emitted into the atmosphere by a multiplicity of sources -- including power plants, vehicular circulation, meat cooking operations and biomass burning -- or secondarily produced by photochemical atmosphere reactions from both biogenic and anthropogenic precursors [1,2].

This paper describes the development of a GC-MS procedure for the simultaneous analysis of several WSOCs with a wide range of water solubility, including carboxylic acids and sugars. The response surface methodology (RSM) including central composite design (CCD) was applied to optimize solvent extraction: the factors considered were the solvent type (characterized by  $p'$  parameter) and volume (10-20 ml). On the basis of RSM, the optimum extraction solvent was a mixture of metane:dichlorometane (90:10) using a volume of 10 ml.

To validate the optimized conditions, a comparative study was performed towards the aqueous extraction, as reference solvent for water-soluble components, for blank filters spiked with standard solutions of WSOCs, and real PM samples.

The optimized procedure provides the low detection limits ( $\leq 2 \text{ ngm}^{-3}$ ) and the good reproducibility ( $\text{RSD}\% \leq 13\%$ ) required by environmental monitoring of chemical markers of atmospheric processes.

In addition, the suitability of the optimized procedure was verified by application to PM filters collected under different conditions, i.e., different seasons (summer vs. winter) and different sampling sites (urban vs. rural).

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## THE COMPETITIVE ADSORPTION OF POLLUTANTS ONTO ZEOLITES

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Human beings are exposed to elevated levels of a wide spectrum of VOCs, many of which have been found to be toxic and potentially carcinogenic. Removal of these organic contaminants from water and wastewater has been achieved using several treatment technologies, such as advanced oxidation processes, air stripping, reverse osmosis, ultrafiltration and adsorption. Recently, it has demonstrated that adsorption technologies is an efficient and versatile methods for water treatment. In particular, hydrophobic zeolites are able to effectively adsorb molecules against which zero-valent iron (ZVI) or granular activated carbon (GAC) are totally ineffective [1-2]. Zeolites are a class of molecular sieves that have a well-defined micropore dimensions and composition in a rigid crystal lattice. These materials are stable at high temperature and have good adsorption capacity.

In this work, combined diffractometric, thermogravimetric and gas chromatographic techniques were employed to study the adsorption process for a mixture of contaminants in the ppb and ppm range. The results obtained for a single component adsorption on hydrophobic zeolites were used as a basis for studying the adsorption process of mixtures of pollutants on zeolites. ZSM-5 was chosen as adsorbent material since previous studies on single components demonstrated that this zeolite has good adsorption capacities and affinity for these compounds even at low concentration levels [3]. In multicomponent equilibria, different molecules can compete for access to the adsorption sites, and those from more strongly adsorbed compounds tend to exclude others. The amount of any component adsorbed at equilibrium depends on the concentration of all the other components in the solution.

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## HEAVY METALS STRESSING ALTERS COMPOSITION OF MEMBRANE LIPIDS OF PHOTOSYNTHETIC *RHODOBACTER SPHAEROIDES* BACTERIA. A TLC MALDI-TOF MS STUDY

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Purple non-sulphur bacteria such as *Rhodobacter sphaeroides* are largely used in bioremediation processes for the degradation of pollutants from contaminated environments [1]. To date, no studies have focused on the relationship between membrane lipid compositions and stressed growth conditions in the presence of nonessential and potentially highly toxic heavy metals. The coupling of thin-layer chromatography (TLC) and matrix-assisted laser desorption ionization with time-of-flight (MALDI-TOF) mass spectrometry (MS) may represent a powerful tool to rapidly monitor the changes in lipid profiles [2,3]. Here we report the analysis of lipid extracts from the membranes of the photosynthetic bacterium *R. sphaeroides* with and without TLC (i.e., direct extract analysis) followed by MALDI-TOF MS. Comparing MS results of lipid extracts, it was noticed that the preliminary separation reduces lipid profile complexity, isolates main interfering species (i.e., bacteriochlorophylls) and decreases suppression effects caused by easily ionizable species such as phosphatidylcholines (PCs). The analysis of lipid extracts in both positive and negative polarities using proper matrices to assist laser desorption ionization, allowed us to identify different components including PCs, phosphatidylethanolamines (PEs), phosphatidylglycerols (PGs), and glycolipids as sulfoquinovosyldiacylglycerols (SQDGs). Finally, the method was applied to investigate membrane lipid modifications of *R. sphaeroides* grown in normal and stressed conditions in the presence of Co(II) or Cr(VI). This study demonstrates the applicability of TLC-MALDI MS strategy to correlate lipid profiling and bacteria response to heavy metal stress.

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## PAHS AND METALS ACCUMULATION IN LEAVES OF URBAN EVERGREEN SHRUBS

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Urban vegetation plays a role in the interception and degradation of air pollutants in cities (1). Trees, shrubs and plants can improve the quality of the air we breathe. Dense screens of trees and shrubs can be used efficiently to provide quieter areas in city parks where people can relax away from the constant background noise of city life. In addition, the foliage of vegetation can trap certain air pollutants, especially air-borne particulates (PM<sub>10</sub>), removing them from the urban atmosphere (2). We report about a study on the concentration of PAHs and metals in the leaves of evergreen shrubs exposed to PM<sub>10</sub>. Analyses were performed on adult specimens of the evergreen *Viburnum lucidum*, *Photinia x fraserii*, *Laurus nobilis*, *Ligustrum japonicum*, *Ilex aquifolium* and *Elaeagnus x ebbingei*, transplanted in November 2009 and sampled in several seasons. Data interpretation can benefit from ultrastructural analysis of foliar surface by SEM.

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## POSITIVE MATRIX FACTORIZATION ON AEROSOL MONITORING DATA IN THE CHAIR PRODUCTION DISTRICT OF FRIULI VENEZIA GIULIA REGION: SOURCE IDENTIFICATION AND SMALL SCALE HETEROGENEITY

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In order to assess the possible relevance on particulate composition of emissions from an incinerator serving a furniture production district in Manzano (Ud), two sampling sites were identified, respectively downwind and upwind to the plant, by means of the CalPuff computational code (1) for modeling the particulate dispersion, considering local orography and meteorology. Two Hydra samplers from FAI Instruments were positioned at the selected sites, allowing the collection of daily PM<sub>10</sub> on quartz filters between February and April 2010. Analytical determinations of OC, EC, WSOC, WINSOC, TC, thirteen PAHs (including benzo[a]pyrene), Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, levoglucosan (2), As, Cd, Ni, Pb were performed. The PM<sub>10</sub> concentration is very similar at the two sites, located 3 km apart, but chemical composition is not perfectly overlapping. A source apportionment study has been performed for the two sites, pointing at the relevance of each source in each sampled day, by Positive Matrix Factorization (3). An agricultural source (ammonia nitrate) was identified, as well as biomass combustion, crustal/dust resuspension (Ca<sup>2+</sup>, Pb/ Na<sup>+</sup>, Cl<sup>-</sup>) and sulfate sources. Unexpectedly, the factor associated to biomass burning, related to EC, BaP, K and levoglucosan, is more relevant at the site upwind from the considered incinerator. The contribution of agricultural activities to the local aerosol is evident and it is highlighted by the “ammonia nitrate” factor but also by the presence of a herbicide in the particulate matter (4), which is one hundred times higher at the downwind site than upwind.

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## **INFLUENCE OF SECONDARY PREPARATIVE PARAMETERS AND AGING EFFECTS ON PLGA PARTICLE SIZE DISTRIBUTION: A SEDIMENTATION FIELD FLOW FRACTIONATION INVESTIGATION**

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Poly(lactic-co-glycolic acid) (PLGA) is a well-characterized polymer from the ester family, widely used in the biomedical industry since its degradation byproducts are non toxic. As biodegradable colloidal particle, PLGA is an excellent delivery carrier for drugs, genes, proteins and various other macromolecules because it shows high stability, has high carrier capacity, can feasibly incorporate both hydrophilic and hydrophobic substances, and offers various feasible routes of administration. Several methods are currently employed to formulate PLGA particles with the smallest possible sizes and maximum stability for pharmaceutical applications

In this project PLGA particles, in the 200-400 nm size range, were prepared by nanoprecipitation and single emulsion (or solvent evaporation) methods in order to achieve particles which can be stable in the long run, that have appropriate dimensions for injectable uses and that disperse themselves well in aqueous media, a key requirement for uses as vehicles to induce in vivo drug targeting. Different concentrations of polymer and stabilizing (Pluronic® F68) were tested in order to identify the best conditions for making PLGA particles of suitable size, stable in time, to be used as carriers for brain targeting drugs.

The particles with the best characteristics for delivery system design were those formulated by nanoprecipitation with an organic/water phase ratio of 2/30, a polymer concentration of 25 mg/mL and a surfactant concentration of 0.83 mg/mL; their surface charge was reasonably negative (~ -27 mV) and the average size of the almost monodisperse population was roughly 250 nm.

Particle characterization was accomplished by using SEM to check the morphology, calculating the surface charge through  $\zeta$ -potential measurements and determining the average sizes and particle size distributions (PSDs), the latter achieved by both PCS (photon correlation spectroscopy) and SdFFF (sedimentation field flow fractionation). SdFFF, the technique considered more reliable than PCS in describing the possible PSD modifications was used to investigate the effects three months of storage at 4 °C had on the lyophilized particles.

## HEAVY METALS POLLUTION: IDENTIFICATION AND QUANTIFICATION IN STRATEGIC ALPINE AREAS

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The glaciers due to distance from densely populated and low biological activity are considered a privileged observatory for research on environmental control and global changes made in recent years since they are the largest environmental memory on the planet (1).

The presence of pollutants in these zones is generally due to atmospheric circulation that transports them from industrialised areas to those farthest.

The chemicals interest include anthropogenic organic substances (cfc, pcb and pesticides), natural (humic and fulvic acids), chemicals (heavy metals) and radionuclides. Toxic heavy metals are released into the atmosphere through natural or anthropogenic processes and can be good indicators for the assessment of human environmental contamination.

The present study focuses on the determination by ICP-MS of the metals both fundamental for human health and strictly connected with environmental pollution. This kind of screening is useful also as preliminary study in order to evaluate if the chosen mountain sites and variables are suitable for a more wide investigation to obtain a monitoring of the seasonal profile of the heavy metals of the last decade (2).

In this work, fresh snow samples from first layer of the glacier were collected from Monte Rosa, Monte Cervino, Monte Bianco e Gran Paradiso in order to evaluate the concentrations of metals, generally present at ppb levels; moreover on these mountains two series of samples were collect: one in a site characterized by a high attendance of tourists (such as skiers, mountain huts) the second in a remote area, where human presence is mostly due to mountaineers who choose less crowded climbing routes. For each sites of the four mountains investigated 5 points were sampled.

The four mountains investigated, due to their geographical position, cover different environmental situations.

All results are correlated thanks to the chemometric treatment of pattern recognition techniques (as Principal Component Analysis) to point out significative grouping of samples with the respectively variables.

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## **DETERMINATION OF INORGANIC POLLUTANTS MOBILITY IN DREDGED SEDIMENT**

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Maintenance, construction and remedial works, within harbor areas imply the production of millions cubic meters of dredged sediment that must be managed. Due to the high marine and terrestrial environmental impacts, both national and mainly international tendency was towards the promotion of alternative management options, furthermore has been slowly introduced the concept of sediment as “resource” instead of “waste”.

Today's there are available several technologies for sediment treatment. A deeply knowledge of chemical and physical properties of the contaminants and the matrix is essential to estimate the economic and ecological impacts of a management strategy (1).

Our study is focused on the investigation of heavy metals mobility in harbor sediments before and after mechanical separation by means a soil washing treatment.

Beside the analysis of total metals content in the whole samples, were determined their release in water under singular experimental condition. Two different leaching tests were carried out: i) pH-stat leaching test CEN/TS 14997; ii) up-flow percolation test CEN/TS 14405. The first leaching test provides information about the influence of environmental pH on solubility of pollutants. The second leaching test is useful to simulate what happens when a waste is stored upland and the water goes throw the material in a sort of piston flow mode, giving information about which mechanisms govern the release of each contaminants.

This preliminary research, other an assessment of soil washing efficiency like a sediment decontamination treatment, shows that many heavy metals increase their mobility in the separated fine fraction compared to the whole sediment.

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## DETERMINATION OF Nb AND Ta IN SEAWATER BY ICP-MS TECHNIQUE: METHOD VALIDATION AND EVALUATION OF MEASUREMENT UNCERTAINTY

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Full informations on trace metals in seawater are important for understanding the marine system. Although they constitute only a small fraction, they provide informations, for example, of geochemical and geological interest. The change in concentration of many of these trace elements, in different geochemical systems, are much larger and significant than the variations of the major components. Nb and Ta, as well as rare earths (Y, Lanthanoids) and high strength field elements (Zr and Hf), are often more sensitive indicators and therefore are key elements of specific processes. In seawater the Nb and Ta dominant forms are respectively  $\text{Nb}(\text{OH})_6^-$  e  $\text{Ta}(\text{OH})_6^-$ . The determination of these elements in aqueous systems and in particular in natural seawater, presents two major problems: first of all the concentration in the order of the pmol/kg [1-2], which requires the use of a sensitive instrument with a very low detection limit and, secondly, the high salinity of the matrix which can seriously interfere with the analytical determinations. For simultaneous determination of Nb and Ta in seawater a coprecipitation with  $\text{Fe}(\text{OH})_3$  with ICP-MS measurements method has been carried out. This has been a development and extension of a validated Zr, Hf and YLOID method [3] The analysis for the method validation was carried out on a spiked natural seawater samples (5-50 ng/L in Nb e Ta) so to verify the efficiency and the reliability of the method itself. Estimation of composed uncertainty associated to measurements was evaluated with a rigorous metrological approach and expressed in terms of precision, recovery, reference materials and instrumental calibration uncertainty. The results obtained show that the method used is quite satisfactory and precise. The percent coefficient of variation (CVr%) at both concentration levels studied falls within the predetermined limit (45%). The ICP-MS determination of the analytes studied is confirmed extremely sensitive and allows to reach extremely low detection limits, with good repeatability, even under routine conditions of a "normal" chemical laboratory.

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## SIMULTANEOUS DETERMINATION OF FOURTEEN CYANOTOXINS BY LIQUID-CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY IN THE DRINKING WATER CHAIN: MONITORING OF AN ITALIAN BASIN.

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Microcystins (MCs), anatoxin-a (ANA-a) and cylindrospermopsin (CYN) are algal toxins produced by cyanobacteria widespread in eutrophic freshwater. These cyanotoxins are different from chemical and toxicological point of view but all responsible for acute and chronic poisoning in animals and humans.

This study aimed to investigate the comprehensive distribution of cyanotoxins (peptide microcystins, alkaloid cylindrospermopsin and anatoxin-a) in Vico Lake (Lazio, Italy), colonized by cyanobacteria *Planktothrix rubescens* and *Aphanizomenon ovalispermum*.

For this purposes, two different, specific and sensitive procedures for determining cyanotoxins in water samples have been employed.

An analytical method based on solid phase extraction followed by detection with liquid chromatography–tandem mass spectrometry (LC-MS/MS) was developed for the simultaneous determination of 12 variants of MCs.

On the other hand, in order to analyze CYN and ANA-a toxins, 100 µL of the filtered water sample were directly injected in the LC-MS/MS apparatus.

Both analytical protocols were validated in terms of sensitivity, selectivity, repeatability, reproducibility, robustness and detection limit, in compliance with the Italian DL 31/2001. The performance and reliability of the method was proven to be adequate for the purposes, with LODs in the range of 0.002-0.017 µg/L for all MCs and 0.08 µg/L and 0.2 µg/L for CYN and ANA-a respectively.

Over twenty-seven months (March 2010-June 2012), an intensive monitoring program of the source water caught by the Vico Lake, as well as of drinking water taken from the Water Treatment Plant and distribution system was implemented to assess the risk on cyanobacteria and their toxins for the served municipality (Caprarola, VT).

The MCs detected in the sample of raw water were [D-Asp3]-MC-RR, [D-Asp3]-MC-LR and MC-RR with maximum concentration of 3.600, 0.237 and 0.100 µg/L respectively. Other toxins (MC-LA, MC-LY, MC-LF, MC-LW), were recorded at levels lower than 0.055 µg/L.

In the final treated water the presence of MCs was detected in not significant concentrations, reaching maximum values of 0.300 µg/L as a sum of all selected congeners. CYN was sporadically observed through the entire drinking water chain, in raw, treated and distributed water from the Vico basin, with maximum concentration of 0.552, 0.187 and 0.167 µg/L respectively. To the best of our knowledge this is the first evidence of CYN in the Vico lake. Conversely, ANA-a was never detected in monitored areas.

Despite the substantial contamination of the raw water, the risk related to human consumption of drinking water was negligible, on account of the low concentration of MCs in tap water, as compared to the guideline value of 1 µg/L set by the WHO.

## INFLUENCE OF NITROGEN SPECIATION ON THE TOTAL NITROGEN MEASUREMENT IN WATERS BY DIFFERENT ANALYTICAL TECHNIQUES

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Analytical methodologies for the determination of Total Dissolved Nitrogen (TDN) are based on a conversion step able to transform all the nitrogen species into a defined compound that is then quantified. Nitrogen is a key nutrient in natural waters and accurate TDN measurements are relevant to assess the status of water bodies, local and global nutrient cycling as well as the efficacy of water remediation treatments (1,2). A crucial requirement is the quantitative recovery of all organic and inorganic nitrogen species during the conversion step. Absolute recovery assessment of the Dissolved Organic Nitrogen (DON) fraction is impracticable due to the sheer complexity of the Dissolved Organic Matter (DOM). Systematic studies concerning its dependence on organic N speciation and on the nature of the conversion step are scarce. Quantitative recoveries were demonstrated only for a limited selection of organic compounds. In this work, the N recoveries of two widely employed analytical methodologies for the determination of TDN (High Temperature Catalytic Oxidation, HTCO, and Persulfate Digestion, PD) were assessed on a set of selected nitrogen-containing compounds, representative of the structures of both DOM and common contaminants. Azo dyes give almost no recovery with either method. Partial recovery with both methods is showed by organic compounds containing triazole rings. S-triazine compounds, guanine and uric acid give satisfactory recoveries only with HTCO. The results show that in many instances the TDN measurements give systematically low results depending on N speciation. TDN data of polluted and waste waters must be considered with care. The estimation of dissolved nitrogen fluxes and pools from TDN measurements can be affected by uncertainties larger than previously thought.

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## SPECIATION OF IODINE AND BROMINE IN ICE AT PICOGRAM FOR GRAM LEVEL

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Iodine and bromine species participate in key atmospheric reactions including the formation of cloud condensation nuclei and ozone depletion. Here we present a new approach for the determinations of inorganic iodine and bromine species at picogram for gram level in Antarctic ice core samples. Samples have been measured by coupling high performance liquid chromatography (HPLC), with ion chromatography (IC) using an IONPAC® AS16 Analytical Column and inductively coupled plasma mass spectrometry (ICP-MS). The procedure for the determination of I and Br species by HPLC-IC-ICP-MS is based on a system of two valve-controlled loops one which handles the NaOH eluent (valve V1) and the other which handles the sample (valve V2) allows the use of aggressive eluents with a standard HPLC pump. This analytical approach could be adopted for many HPLC applications where aggressive eluents are required. Detection limits for I and Br species were 5 pg g<sup>-1</sup> for I<sup>-</sup>, 7 pg g<sup>-1</sup> for IO<sub>3</sub><sup>-</sup>, and 9 pg g<sup>-1</sup> for Br<sup>-</sup> with an uncertainty of less than 2.5% for all considered species. The interglacial ice core results reported here indicate that both I and Br are present at concentrations in the pg g<sup>-1</sup> range but only the inorganic species are present. Interglacial ice core results indicate bromide as the only bromine species while iodine was present only in I<sup>-</sup> form and displayed greater variability. Iodate was not present above detection limits even though it is believed to be the most stable form

## **ANALYSIS OF CONTEMPORARY ART MATERIALS: SYNTHETIC PIGMENTS AND BINDERS OF "RI DE POMME" BY JULIAN SCHNABEL AT THE PECCI MUSEUM, PRATO**

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From the beginning of XX century, new classes of synthetic organic pigments and paint binders became available on the market. Since then, artists and restorers started to introduce these industrial products in artworks. The range of materials and formulations that can be encountered in contemporary art is extremely wide and in constant evolution. Consequently, long term behaviour and the ageing pathways are not completely known for most of them. The knowledge of the constituents materials of contemporary artworks, and of their degradation processes, is extremely relevant for the selection of preventive conservation conditions, and for the choice of restoration methodologies and materials.

The identification and the assessment of the state of conservation of synthetic polymers requires the application of focused analytical strategies, that combine non-invasive approaches with detailed molecular analysis by chromatographic techniques combined with mass spectrometry.

Here we report preliminary investigations on a painting by Julian Schnabel, *Ri de Pomme* (1988), owned by the Pecci Museum for Contemporary Art (Prato, Italy). Non-invasive in situ investigations by FORS (fiber optics reflectance spectroscopy) allowed us to differentiate between the original and restored areas of the paint layer. Selected microsamples have been submitted to analysis by GC/MS (gas chromatography/mass spectrometry) coupled with analytical pyrolysis, for the identification of the binder used by the artist and of restoration materials.

The study of Julian Schnabel painting materials represents an excellent case study for testing and improving analytical techniques for the identification of synthetic organic pigments and binders. The results permitted to clarify original and restoration materials and to draw conclusions on their state of conservation, highlighting the high level of complexity of scientific investigations on contemporary art .



## GC/MS AND THERMAL ANALYSIS IN THE CHARACTERIZATION OF WOOD AND BARK PITCH REPLICA FOR ARCHAEOLOGICAL PURPOSES

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To achieve a proper scenario on the bio-molecular markers, which allow us to identify archaeological tar and pitch, the chemical characterization of reference materials is an essential tool. In this view, a collection of more than 30 tar and pitch was prepared from wood and barks of various deciduous trees (mainly from genus *Betula*) and conifers (mainly from genus *Pinus*). Tar and pitch were obtained following two different methodological procedures: single and double pot distillation. Chemical investigation on such a collection was performed using both an analytical procedure based on gas chromatography/mass spectrometry (GC/MS) and thermal analysis (TGA thermo gravimetry coupled with IR for evolved gas analysis, EGA).

This study also aims at comparing the performances and the complementarity of both techniques in the analyses of pitch and tar. From one side, GC/MS has been already applied with great achievement in the characterization of organic residues from archaeological findings. The analytical procedure based on GC/MS will be used to assess the molecular composition of the replica and to identify a series of species acting as markers of botanical origin, of technological manipulation and eventually of degradation. On the other, TGA will be used to characterize the thermal and thermo-oxidative decomposition of the selected pitch on the basis of their different compositions. The analysis of the gas evolved by the samples during heating may help not only in understanding the thermal degradation pathways, but also in monitoring the presence/release of specific substances. The results could be useful to identify the thermal changes induced on pitch by heat exposure and to reconstruct the thermal history of archaeological samples.

## TAG PROFILING BY HPLC/MS FOR THE IDENTIFICATION OF LIPIDS IN HISTORICAL SAMPLES

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The identification of the source of lipid materials in archaeological or historical objects can yield important archaeometric information and contribute to the knowledge of past technologies and to the planning of conservation strategies. Lipids can be encountered as paint media or as residues of content in vessels. In particular oils and fats were used not only as food but also as illuminants or ingredients of cosmetics and medicines.

Identification of the botanical or animal source of lipids represents an analytical challenge at the present state of the art. A recent approach (1), alternative to GC/MS analysis after saponification, is based on the determination of the overall triglycerides (TAGs) profile determined by HPLC/MS. The possibility to separate and determine intact TAGs without any modification of the samples (which can cause loss of information) is the main advantage of this technique (2). In this work we compare two detection systems for the LC analysis of the TAGs profiles: atmospheric pressure chemical ionization with single quadrupole MS (HPLC-APCI/MS) and electrospray ionisation with quadrupole/time of flight tandem MS (HPLC-ESI-Q/TOF). In both instrumental assets positive-ion ionization was used, and the recognition of TAGs was based on evaluations of the peaks in extracted ion chromatograms of the parent ion mass ( $[M+H]^+$  for APCI,  $[M+Na]^+$  for ESI) and of known fragments deriving from each TAG. The use of the two detection systems appeared complementary. On the one hand, the fragments are directly formed in APCI due to the excess of energy involved in the corona discharge ionization process and thus APCI-MS can be used for qualitative preliminary evaluation of the overall TAG profile in the sample. On the other hand, thanks to its higher mass resolving power and to the availability of tandem MS, ESI-Q-TOF can be used for the ultimate identification and quantification of selected TAG, chosen as biomarkers. We analyzed reference oils and fats, and organic residues found in XVI century historical ointments of Museo Aboca in San Sepolcro (Arezzo). We identified more than 20 species corresponding to DAG, TAG and to high molecular weight condensation products. Evaluation of the data included application of principal components analysis (PCA) (3).

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## CHARACTERISATION OF LOW MOLECULAR WEIGHT PAINT VARNISHES BY ANALYTICAL TECHNIQUES BASED ON MASS SPECTROMETRY

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The diffusion of low molecular resins in conservation is due to the fact that they have similar optical and physical properties to those of traditional natural terpenoid resins, such as dammar and mastic, but are believed to be more stable under ageing. The low molecular weight guarantees a low viscosity of the varnish solution, which ensures the obtainment of a smooth varnish film after the evaporation of the solvent, and, thus, a proper colour saturation and gloss of the paint<sup>1</sup>. The quality of the film is also guaranteed by the fact that these low molecular resins have a refraction index very similar to that of traditional paint layers, ensuring a reduced light scattering at the varnish-paint layer interface<sup>2</sup>. In addition to these positive aesthetical qualities, another advantage offered by low molecular weight and low-polarity is the possibility to apply and remove the varnish without using polar solvents, which can be extremely aggressive to the paint layers underneath. On the other hand, the chemical behaviour of this polymers, especially under ageing, is not well known.

We performed the analytical characterization of three low molecular weight resins widely used as paint varnishes: the hydrocarbon resin Regalrez 1094, the aldehyde resin Laropal A81, and the ketone resin MS2A.

Analytical pyrolysis coupled with GC/MS (Py-GC/MS), direct exposure-mass spectrometry (DE-MS) and liquid chromatography coupled with high resolution mass spectrometry (HPLC-ESI-Q/ToF) were used to characterize commercial Regalrez 1094, Laropal A81, and MS2A, in order to elucidate their chemical structure, and to collect pyrolytic and mass spectrometric profiles suitable for their identification in unknown samples.

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## MONITORING THE CONSERVATION OF CULTURAL HERITAGE WITH IMAGING SPECTROSCOPY: HOW TO DISTINGUISH PHYSICAL-CHEMICAL CHANGES FROM SHAPE CHANGES

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In the last years the targets of the research in conservation sciences has concerned the development of monitoring methodologies that not only do not damage the object of art, leaving it unchanged for successive examinations, but that are also able to analyze the entire artifact at the same time. Imaging spectroscopy techniques, and in particular a LED Multispectral Imaging method coupled with multivariate analysis, has provided an excellent solution to these **problems[ref]**. This approach is based on the comparison of the artifacts over time in order to monitoring the conservation state and to alert conservators in case dangerous situations appear. The proposed procedure is a pixel-by-pixel strategy, and it is dependent on the image alignment, which is a crucial step. Some cultural heritage objects, like parchments and other organic supports, are subjected to shape deformations over the time.

This paper will present here a method to distinguish the information about physical-chemical changes (reflectance) from shape deformations using image warping algorithms. The separation of these two sources of information is mandatory in order to avoid confusing physical-chemical changes, which are strictly related to the reflectance of the artifacts, with shape changes. Image warping algorithms for the registration of images taken at different times allow to separate reflectance changes from shape changes: deformation grids, masks and deformation fields are very useful tools to extract new information from the artifact that could lead to misinterpretation of the results. Physical-chemical changes and shape deformations implies different restorative approaches.

## APPLICATION OF LC-MS AND LC-MS-MS TO THE ANALYSIS OF PHOTO-DECOMPOSED CRYSTAL VIOLET IN THE INVESTIGATION OF CULTURAL HERITAGE MATERIALS AGING

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In this work the accurate LC-UV-Vis, LC-MS, and LC-MS-MS analysis of the photo-degradation products of crystal violet, CV (1), a widely used dye in ancient writings and drawings, is reported. On-line photodiode array detector enabled simultaneous UV-Vis spectra acquisition. Many degradation compounds were identified through their exact mass (2 ppm accuracy) and MS-MS technique. In particular, all CV demethylated products, demethylated Michler's ketone (2,3) and particularly some oxygen containing substances, such as N-oxides (4) were identified. Fragmentation products are all justified by the proposed fragmentation scheme, in term of precursor exact mass and isotopic profile, characteristic losses in fragmentation and rebuilt structure formula. In particular, we documented the presence of N-imido oxides and hydroxylamine derivates, never reported before, together with the demethylated derivatives of the studied dyes. All these compounds, although at trace level in our samples, contribute to colour and intensity modification of ancient artifacts. In particular, demethylation of CV by UV light leads to formation of compounds absorbing at shorter wave-lengths (blue shift) or no-absorbing in visible range (yellow-colourless) appearing reddish-brown (5). This phenomenon justifies drawings appearing grey or brown on the old yellow paper, when CV-based inks or paints were used. The final aim is to recognize provenance of cultural heritage manufactures and their collocation in time.

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## A COMPARATIVE STUDY OF ORGANIC/NANOPARTICLES COATINGS FOR ENHANCED STONE PROTECTION

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Conservation of historical buildings is an important issue; the environmental conditions seriously affect the monumental stones. The protection of cultural heritage buildings and monuments by surface treatment with polymers is a common practice due to their ability to form a protective layer on the monumental surface as well as to control the transport of different fluids from the surface to the inner part [1–3].

Moreover, in the last few years, nanomaterials have been frequently applied for restoration and conservation of artworks. It is worth noting that inorganic nano-oxides, such as silica and titania, improve the performances of materials used in conservation field [4]. In particular, the application of photocatalytic coatings on stone has been investigated for providing surface protection and self-cleaning properties [5].

In this work three different substrates were used: Carrara/Botticino marbles, and Angera stone. Commercially available siloxanes and acrylic polymers were used as protective agents to improve the hydrophobicity features of the different tested materials. Then the conservation effectiveness of inorganic nanoparticles dispersions was evaluated when applied on the porous stone substrates. The strengthening effect of the nanoparticles-based treatments is compared to that exhibited by the well-known consolidant polymers.

Morphological (SEM-EDS), structural (XRD), thermal (TGA) and spectroscopic (FTIR and DRS) analyses were carried out on coated and uncoated stones to establish the changes of appearance, color, and water absorption. Static/dynamic contact angle measurements and surface free energy determinations were adopted to evaluate the final wettability and self-cleaning properties before and after UV-light exposure and artificial ageing.

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## MULTI-SPECTROSCOPIC APPROACH TO EXPLORE THE TECHNOLOGICAL FEATURES OF MEDIEVAL GILDED AND ENAMELLED GLASSES FROM MELFI (PZ)

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Gilded and enamelled glasses of Islamic style, coming from a 13<sup>th</sup> century landfill in Melfi castle, a Swabian emperor Frederick II fortress, were subjected to a multi-techniques approach in order to explore the complex and very fascinating ancient production technology of gilding and enamelling on glass. Non-destructive  $\mu$ -Raman spectroscopy was employed on the most important and well-preserved objects, optical (OM) and electron (SEM) microscopies were used to investigate the sections stratigraphy of tiny fragments sampled from the borders of the already damaged objects. In order to provide the chemical analyses of the bodies and the enamels, energy dispersive X-rays spectroscopy (EDS) and X-rays photoelectron spectroscopy (XPS) were also employed. The body of the objects proved to be made of silica-soda-lime glass, while the enamels of lead-rich glass (“soft enamels”) and coloured by lapis lazuli and cobalt for blue, hematite and minium for red, lead-tin yellow for green and calcium phosphate for white. The gilding was found to be applied on a red enamel basis. The presence of carbon inside the gildings and the detection of two different gold signals by XPS suggested the hypothesis of the use of the so-called “liquid gold”. This study gave thus an important contribution to the understanding of the production of this class of rare and precious objects, also confirming that the materials and technological procedures are consistent with the Islamic tradition, probably due to the presence of Islamic artisans at the court of Frederick II.

## REMOVAL OF *GRAFFITI* FROM APULIAN LIMESTONE BY LASER CLEANING

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In the last twenty years great attention has been paid find the more suitable way to remove graffiti, responsible of the esthetic defacement of stone monuments. Chemical, mechanical and physical treatment have been developed. Among these, laser ablation has acquired a considerable role. The present work aims to find a way to evaluate the efficiency of laser ablation on Apulian limestone covered by spray varnishes. Objects were subjected to diagnostic investigation before and after laser cleaning to obtain indispensable information to plan and perform cleaning procedures and to check the efficacy of the procedures.

Four different colored varnishes (white, red, black and yellow) and nine Apulian limestone have been investigated. Py-GS-MS and  $\mu$ -Raman have been used to characterize binders and pigments of the varnishes, optical microscopy and profilometry to identify petrographic and surface characteristics of limestones. Laser cleaning tests were performed on the different substrates in order to evaluate the efficiency of the cleaning in funzione di: roughness of limestone surface, porosity and petrography, nature of pigments and binders of the varnishes. Limestones have been artificially aged for thirty days exposing each sample to twelve-hour cycles of sun light (T=65°C), freezing (T=-25°) and rain (T=20°C). After the ageing, samples have been sprayed with the varnishes and left drying for a week. Nd:YAG laser ( $\lambda=1064\text{nm}$ ) has been used both in Short Free Running mode (pulse duration from 40 to 110  $\mu\text{s}$ ) and Q-Switched mode (pulse duration 8 ns). Laser tests has been led both in dry and in wet conditions. Damage thresholds have been estimated on the natural and aged limestone sample in order to evaluate the operating fluence range. Colorimetric analyses and microscopy observations have been led on the untreated, aged and treated samples in order to evaluate the cleaning efficiency. Comparison have been done with chemical cleaning tests. Good results have been obtained for laser cleaning of black and red varnishes both on the high-porous and on the low-porous limestones. On the contrary, laser cleaning has left residues of white varnish on both the limestones and for any fluence. Moreover, in some cases the white has turned into grey. Only for white-spried low-porous limestone chemical cleaning efficiency is better than the laser one.



## MULTI ELEMENTAL ANALYSIS OF ROMAN POTTERY FROM NORTHEASTERN ITALY BY INDUCTIVELY COUPLED PLASMA – OPTICAL EMISSION SPECTROSCOPY (ICP-OES) AND MASS SPECTROMETRY (ICP-MS)

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Chemical characterization has been carried out on 200 ceramic samples belonging to different classes of pottery (grey, coarse and *semidepurata*; I century B.C.–II century A.D.) coming from two archaeological sites in the area of Millepertiche (Veneto, Italy). The aims of the present study are to evaluate the differences in the pattern distribution of the sherds between the two sites and to better understand the ceramic typology for classification studies(1, 2). The archaeological interest of the study is related to the sites location since they are situated on the historical *Via Annia*. The *Via Annia* was an important vector for the circulation of material of different provenance, as it was built in the Roman period to connect northeastern Italy with Rome and the rest of the road system in the peninsula.

For these purposes multielemental analysis has been carried out. After sample digestion in a microwave oven, the ceramic chemical composition in terms of major (Na, Mg, Al, Si, K, Ca and Fe) and minor elements (Sc, V, Cr, Mn, Co, Ni, Cu, Zn and Pb) was determined by means of inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) (3, 4, 5). The most interesting major elements are Si, Ca and Fe, with concentrations that ranged respectively from  $11 \cdot 10^4$  to  $40 \cdot 10^4$  ppm, from  $3 \cdot 10^3$  to  $11 \cdot 10^4$  ppm, and from  $4 \cdot 10^3$  to  $11 \cdot 10^4$  ppm. Chemometric techniques such as principal component analysis (PCA) and cluster analysis have been applied to the complex dataset for extracting useful information in order to identify similarities and differences among ceramic samples. The results demonstrate that the different chemical compositions could be related both to the provenance from the two sites and to different pottery typology and fabrication.

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## DEPOSITION IN ST. MARK'S BASILICA OF VENICE

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Atmospheric pollutants may cause damage to monuments and historical buildings. Besides air contaminants, soluble salts are also responsible for stone deterioration and decay in outdoor and indoor monuments(1,2,3,4). The problem of how to conserve works of arts thus requires a deep knowledge of contaminants' concentration and distribution inside buildings. In this work, water-soluble ions inside St. Mark's Basilica in Venice were studied, with the aim of understanding their principal source and distribution inside the building. With the aid of FT-IR and SEM analysis, the interaction between ions and surface's material was also investigated.

Ion-chromatographic analysis of depositions highlighted a large amount of "deteriorating agents" such as sulphates and chlorides. A possible source in the innermost area of the Basilica has been found for formates and nitrates. On the contrary, a decrease of chloride, from the entrance to the innermost area, has been found, which indicate the source is outside the building.

It is emphasized that different contaminants behave differently on different material, and the effect of pollution inside churches and monuments is not easy to predict. Wood and brick seem to react differently than stone and mortar to the damaging action of salts and pollutants.

The present work should be considered a useful tool for the future preservation of St. Mark's Basilica in Venice.

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## ANALYSIS OF EGG-BASED MODEL WALL PAINTINGS USING AN INNOVATIVE COMBINED DOT-ELISA AND UPLC-BASED APPROACH

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The chemical analysis of egg-based wall paintings – *mezzo fresco* technique – is an interesting topic in the field of organic binders characterisation. A revised procedure for a Dot-Enzyme-Linked ImmunoSorbent Assay (Dot-ELISA) able to detect protein components of egg-based wall paintings was reported. The new Dot-ELISA procedure succeeded in maximizing the staining colour acting on the temperature during the staining reaction. Quantification of the colour intensity by visible reflectance spectroscopy allowed to obtain a straight line of the protein concentration *versus* the reflectance in the wavelength range 380-780 nm. The modified Dot-ELISA protocol was proposed as a semi-quantitative analytical method for the characterisation of protein binders in egg-based paintings. In order to evaluate its performance, the method was first applied to standard samples (ovalbumin, whole egg, egg white), then to model specimens, and finally on real samples (Giotto’s wall paintings). Moreover, the amino acids analysis carried out by the innovative AccQ•Tag™ Ultra Performance Liquid Chromatography was applied to both the standard and model samples and the results were compared with those of the Dot-ELISA tests. In particular, after protein hydrolysis (24h, 114 °C, 6M HCl) of the samples, the amino acid derivatization by 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate allowed a reproducible amino acids analysis. This UPLC AccQ•Tag™ Ultra amino acids analysis revealed itself rapid and reproducible and was applied for the first time to egg-based paintings. Since the painting technique involved the use of egg-based tempera on fresh lime-based mortars, the study enabled to investigate the influence of the alkaline environment on the egg-protein detection by both methods.

## CHARACTERISATION OF THE HISTORICAL MORTARS FROM ARSENAL OF AMALFI

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The Arsenal of Amalfi dates back to XI century, when this town was at the height of its glory as a Maritime Republic. The building is the only surviving example of medieval shipyard in Italy. Its structure consists in 2 large lanes divided by 20 stone pillars; it has been revised more and more times due to natural events, changes in use, renovations.

The mortar samples were taken from walls and pillars during the last conservation work and analysed to study the composition, the building technique and the conservation state.

The components were investigated by the observation of thin sections, x-ray diffraction (XRD), thermal analysis (TG-DSC) and infrared spectroscopy (FTIR) analyses.

Some products of decay phenomena were found, in part due to the proximity of the Arsenal to the sea.

The highlight of the analytical campaign was the determination of organic compounds by FTIR analyses after extraction in solvents. GC-MS investigation confirmed the presence of polysaccharides, most probably a natural gum used as additive.

## DESIGN AND DEVELOPMENT OF A MONITORING SYSTEM FOR LOCALIZED CLEANING OF PAPER MATERIALS: PRELIMINARY RESULTS

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Paper materials are subject to the phenomenon of "natural aging", a spontaneous deterioration due to the organic nature of its components and one which is sometimes aggravated by incorrect cleaning or restoration that further undermines its conservation status over time. Among the most sensitive and important issues in the restoration of a paperwork include: cleaning of the sheets, the estimation of their "health", the optimization of the degree of humidity of the pages, and the removal of the adhesives used which are often a source of degradation. In recent years several techniques, less invasive than the traditional ones, have been developed for surface cleaning of library materials with the objective that they could limit the use of water, not only to protect the integrity of various notes and images included in ancient paper documents, but also to minimize the impact of the solvent on the paper [1]. Among the wet cleaning techniques, one of the most effective tools to minimize the impact of the aqueous solvent is the use of a rigid gel of gellan gum. These gels allow one to simultaneously carry out interventions of wet surface cleaning and washing with a modulation of the supply of water depending on the conservation needs and characteristics of each individual product. This paper proposes using the gellan gel for non-invasive cleaning of targeted, localized areas of paperwork and, through identification and measurement of selected analyte molecules, to achieve a rapid and continuous monitoring of the conservation status of the paper material. In conjunction with application of the gel, electrochemical biosensors were employed to monitor specific molecules which reflect the state of degradation of the material itself [2] as well as the success in removing extraneous elements. For this work, in which we present a model of this approach, glucose has been chosen as the final index of the degradation because it is the main product of the breaking of the  $\beta$ -(1  $\rightarrow$  4) glycoside bond of cellulose, the main component of the paper. It can also be a breakdown product of binding materials. The polymer chains of cellulose may also in fact be ruptured following various degradative phenomena such acid hydrolysis or exposure to the  $\beta$ -glucosidase class of enzymes that are produced by *Aspergillus niger* and *Trichoderma reesei*. These latter organisms utilize the pages of books as both physical substrate and source of nutrients. In this study of a model for this approach, we have demonstrated the cleaning procedure on white filter paper while the process of the cleaning was simultaneously monitored by the electrochemical biosensor to detect glucose. In a second phase, the paper was attacked artificially by the enzyme  $\beta$ -glucosidase in order to simulate degradation. Various parameters affecting the electrochemical measurement of glucose were optimized. Finally the cleaning/monitoring procedure was applied to various kinds of paper that represent the types of material found in paperwork artifacts. The combination of the results obtained has allowed us first to confirm the efficacy and selectivity of the proposed cleaning method and secondly to demonstrate the benefit of its coupling with an electrochemical biosensor that permits the continuous monitoring of the cleaning process.

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## **FTIR MICROSCOPY IN THE NEAR REGION: AN ADVANCED SPECTROSCOPIC APPROACH FOR THE INVESTIGATION OF PAINT CROSS-SECTIONS**

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Over the last decades, near-infrared spectroscopy (NIRS) has emerged as a powerful and versatile analytical technique, suitable for a wide variety of applications. Nevertheless, sporadic applications have been reported in the field of Cultural Heritage and these were mainly related to *in situ* non-invasive analyses.

The present research was aimed at exploiting the near infrared region (between 4000 and 8000  $\text{cm}^{-1}$ ) for the investigation of paint cross-sections, employing a new integrated FTIR infrared microscope in the total reflection mode. Thanks to the acquisition range of the mercury cadmium telluride (MCT) detector, both the mid (MIR) and near (NIR) infrared region were investigated and information provided by each of them was compared.

Sample preparation was optimised in order to control the superficial roughness. In fact, it is known that increasing the diffuse components allows to enhance spectral features in the NIR region. These overtone and combination bands are extremely diagnostic and, deriving by the diffuse components, are not affected by distortion.

Both single point and mapping analyses were performed on standard and real samples in order to provide a stratigraphic localisation of paint materials. In addition, multivariate chemometric methods were applied to the data to extract and interpret the maximum information embedded within spectral features.



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