ISTITUTO SUPERIORE DI SANITÀ

V Mediterranean Basin Conference on Analytical Chemistry

Silvi Marina, Teramo, Italy
24-28 May, 2005

ABSTRACT BOOK

Edited by
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The fifth edition of this Conference, as the past ones, deals with all aspects of analytical chemistry in the countries of the Mediterranean basin. With over one hundred oral and poster presentations and five short courses, an overview is presented on current challenges posed to analytical chemistry in fields as diverse as food safety, environmental protection, biochemical studies, drug characterisation, method innovation and instrumental development. The quest for quality, pivotal to the credibility of analytical information and to its proper use by the decision makers, is highlighted in most presentations and is illustrated in an *ad hoc* session as well as in quality-centred short courses. The variety of issues illustrated and the experimental approaches suggested testify to the wealth of information provided by this Conference along with the progress made so far by analytical sciences.

Key words: Environmental protection, Human health, Mediterranean basin, Analytical chemistry, Quality systems

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La quinta edizione di questo Convegno copre, come i precedenti, tutti gli aspetti della chimica analitica per i paesi che si affacciano sul bacino del Mediterraneo. Con oltre cento presentazioni orali e poster, nonché cinque corsi brevi, la manifestazione fornisce una sintesi sulle problematiche attuali della chimica analitica in campi diversi, come la sicurezza alimentare, la protezione ambientale, gli studi biochimici, l'esame dei farmaci, l'innovazione metodologica e lo sviluppo strumentale. La garanzia di qualità, essenziale per la credibilità dell'informazione analitica e per il suo uso appropriato da parte di chi deve prendere decisioni, è esposta in buona parte delle presentazioni, oltre ad essere illustrata in un sessione ad hoc ed in due corsi brevi. La diversificazione degli argomenti trattati e le soluzioni analitiche proposte costituiscono una dimostrazione della ricchezza di informazioni fornite dal Convegno unitamente al progresso fatto finora nel campo delle scienze analitiche.

Parole chiave: Protezione ambientale, Salute, Bacino del Mediterraneo, Chimica analitica, Sistemi di qualità

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PREFACE

The V Mediterranean Basin Conference on Analytical Chemistry takes place at Silvi Marina (Teramo), Italy, from 24 through 28 May 2005. The past events took place at Córdoba, Spain (1995), Rabat, Morocco (1997), Antalya, Turkey (2000) and Portorož, Slovenia (2002).

The fifth edition of this conference, especially intended for the Mediterranean scientific community, brings together analytical chemists, marine scientists, environmentalists, quality managers, manufacturers and suppliers of equipment and consumables as well as all experimentalists having an interest in obtaining reliable quantitative data for a better protection of human health and the environment.

Pre-conference short courses

Short course 1

APPLICATION OF INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION AND MASS SPECTROMETRY FOR COMPLIANT ANALYSIS OF WATER, WASTES AND RELATED SOLID ENVIRONMENTAL SAMPLES

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Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) are now widely employed for the compliant determination of trace, minor and major elements in water and wastes - from natural surface, drinking and ground water to industrial effluents and toxic solid wastes. Owing to the excellent analytical characteristics of these instrumental procedures, they are specified in Standard Operating Procedures (SOPs) by regulatory agencies. In this one-day short course the application of ICP-AES and ICP-MS using compliant US EPA 200.5, 200.7, and 200.8 as well as SW 846 6010 and 6020 ICP-AES and ICP-MS procedures are addressed. The course includes aspects of sampling, sample delivery, storage and conventional and microwave-assisted sample preparation. These approaches are discussed in terms of end-user requirements, contamination, sample throughput and cost. The requirements of the various procedures are described using conventional figures of merit such as instrumental and method limits of detection, linear dynamic range and analyte recovery. Emphasis is placed on the systematic correction of spectroscopic and non-spectroscopic interference effects such as those that occur in the sample introduction system, in the plasma and in the plasma-MS interface. An account is made of the various Quality Assurance/Quality Control (QA/QC) validation procedures such as addition method, spiking, certified reference materials and proficiency testing. An appraisal of these procedures will be performed in terms of the requirements of the ISO/IEC 17025 accreditation standard and the need to develop statements of analytical uncertainty. Participants are encouraged to discuss their concerns and applications.

Short course 2

SPECIATION ANALYSIS FOR ENVIRONMENTAL, FOOD AND INDUSTRIAL APPLICATIONS

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Metal speciation is gaining increasing use and applications in a wide variety of fields. It is well understood nowadays that it is essential to have the chemical formulation of the metals of interest to correctly assess their fate, impact and risk assessment in all traditional compartments where inorganic analysis is involved. The last 20 years have seen considerable efforts of the academic and industrial community to identify, rationalise, simplify and promote simple and effective analytical solutions to improve our understanding of metal-related issues in environmental, food and industrial hygiene as well as in industrial issues. The traditional and classical aspects of the analytical chain have been reinvestigated to sample, extract and detect the analytes of interest.

The objectives of this course are three-fold, *i.e.: i*) position the present state of development of metal species determination (elemental speciation) with respect to traditional analytical procedures and to identify the areas of growing demands; *ii*) to examine and critically assess all the major developments in sample collection, sample preparation and detection (review of the general analytical trends both in separation and detection approaches); *iii*) to address and illustrate with appropriate examples and analytical solutions the practical requirements in different cases selected from the environment, the food and the industrial applications.

The general objectives of this course are to reach the widest possible audience. It aims at helping analysts to identify speciation issues that they will have to deal with and select the most appropriate analytical approaches to solve the problem in a cost-effective manner. This course also provides the attendees with a complete review and state-of-the-art in this domain. This course is open to beginners as well as to advanced analysts. It serves the purpose of informing analysts of the growing demand for elemental species determination, critically reviews the field and the state-of-the-art and helps them to develop appropriate analytical responses to the problems that they will have to face in the future.

Short course 3

UNCERTAINTY OF MEASUREMENT RESULTS

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Measurement uncertainty is an important requirement of the ISO/IEC 17025 accreditation standard requirement. This module explains and demystifies the approach of the ISO-GUM (Guide to expression of uncertainty in measurement) to estimate and report the uncertainty of a measurement result obtained following a specific measurement procedure. A clear description of all steps needed for uncertainty evaluation is presented with the respective examples.

Short course 4

TRACEABILITY OF MEASUREMENT RESULTS

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In this module, various issues concerning traceability of chemical measurement results are addressed. According to VIM, “traceability” means “properties of the result of a measurement of the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties”. Therefore, every link in the traceability chain should consist of comparisons that are measurements in accordance with the above-proposed meanings, which include the validation of the measurement procedure and the use of reference materials. Not all chemical measurements are, or should be, traceable to the mole. Other stated references are accepted as well.

Short course 5

TRACE ELEMENT SPECIATION RESEARCH GOING INTO HETEROATOM-TAGGED PROTEOMICS

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Many known proteins contain metals or semi-metals in their structures. Those elements can be present as the prosthetic group of conjugated proteins (like metalloenzymes) whose function depends on the presence of the metal ion in their structure or just forming stable macromolecular complexes for metal storage and/or transportation through living systems. The characterisation of such structures is of extraordinary importance to asses the biological role of trace elements in living systems. Hence, it is a real must today from both the bioinorganic and the clinical points of view. For this purpose, specific "heteroatom tagged" investigations, using trace element speciation *via* Inductively Coupled Plasma Mass Spectrometry (ICP-MS), can provide valuable species information clearly complementary to classical molecule-selective detection. Thus, the latest analytical strategies to tackle speciation analysis and the state-of-the-art of the instrumentation employed for this purpose are critically reviewed in this course. Hyphenated techniques based on coupling chromatographic separation techniques with ICP-MS detection are now established as the most realistic and potent analytical tools available for real-life speciation analysis. Therefore, the importance, status and potential of metal and semimetals elemental speciation in large bio-compounds using ICP-MS detection are first illustrated. Three different aspects of this field are also illustrated and discussed in detail, *i.e. i*) metallo-complexes (*e.g.*, Al, Fe and V in serum transferrin); *ii*) (semi-metal)-proteins, with Se as a case study (from small metabolites to big biomolecules; and *iii*) ICP-MS for heteroatom detection (non-metals P and S) in proteins. An effort is made to assess the potential of present trace elements speciation knowledge and techniques for "heteroatom-tagged" (*via* ICP-MS) proteomics.

Oral session 1

Fundamentals

Chairperson
P. Caglar

THE BEST-KEPT SECRET IN LIFE SCIENCE SEPARATIONS: FIELD-FLOW FRACTIONATION

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The explosive growth of analytical methods for life science applications demands improved tools with ample application range, resolution and versatility. Methods and technology for Gel and Capillary Electrophoresis (GE, CZE) and for Liquid Chromatography (LC) have rapidly developed to face new analytical challenges in the separation of nucleic acids, proteins and peptides and cell metabolites. Hyphenation of these separation techniques with identification and characterisation methods based on Mass Spectrometry (MS) today represents the most versatile analytical approach to genomics, proteomics and metabolomics.

Parallel to the development of GE, CZE and LC, somewhat slower and less visible has been the development of Field-Flow Fractionation (FFF). FFF is a set of rapid and selective separation tools for macromolecular, nano- and micron-sized analytes. The FFF process fulfils many requirements for life science applications, among which worth mentioning are high selectivity and speed, simultaneous measurement, simplified coupling to other measurement devices, applicability to diverse samples over 15 orders of magnitude in mass-size range, gentleness in separating delicate species and high application flexibility. Despite these outstanding features, the number of FFF references found in the literature through SciFinder® is about two orders of magnitude lower than the total number of LC references.

This lecture describes basics, features and possible constraints that have made FFF the best-kept secret in the life science separations. Progresses in FFF technology, as well as the recent development of coupled techniques combining FFF separation capabilities with specificity and sensibility of well-assessed methods for bioanalysis, such as low-impact MS, flow citometry, immunometry and luminescence detection, are also presented. Sorting and fingerprinting of bacteria for whole-cell vaccine productions, non-invasive and labelless sorting of neoplastic or stem cells, separation of native proteins and enzymes in top-down proteomics and the development of flow-assisted, multianalyte immunoassays are some of the most recent applications that could in future increase FFF penetration into life sciences to hopefully unveil this secret.

GRAVITATIONAL FIELD-FLOW FRACTIONATION WITH FLOW CYTOMETRY FOR SORTING OF IMMATURE CELLS

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The ability to discriminate and quantify distinct populations of cells has become increasingly important with the growing trend to focus biological studies on various cell types for many biomedical applications such as their use both in research and in patient treatment. Flow cytometry may be considered now a set of unique techniques that permit the identification, analysis and purification of cells suspensions based on their expression of specific markers. Cell sorting technology has increasing interest in applications that require isolating of particular types of cells, such as immature cells, for which only few and not specific markers are known. As a consequence, the isolation from a heterogeneous population based on physical properties of a cell of interest represents an important goal in biomedical applications and requires the development of new, non-invasive and tagless separation techniques.

Gravitational Field-Flow Fractionation (GrFFF) is a low-cost and biocompatible flow-assisted separation technique suitable for micron-sized particles, among which cells. The separation, based on the differences in the multi-polydispersity indexes (*e.g.*, differences in morphology, density, membrane rigidity and surface properties), is structured by the interaction of samples with Earth gravity field applied perpendicularly to a mobile phase flushing into a thin (80-250 μm in thickness) channel. The soft retention mechanism and the simple maintenance of sterile conditions allows cells viability to be preserved and eluted cells to be collected for different biomedical applications, such as clinical diagnostics and therapy. In this work we present the use of GrFFF with Flow Activated Cell Sorting (FACS) for the enrichment of Human Hematopoietic Stem Cells (HSCs) from peripheral blood. The transplants of these cells are now routinely used to treat patients with cancers and other blood and immune systems disorders. Samples recovered after an aphaeresis procedure from circulating blood able to isolate white cells, were injected into a GrFFF channel. Fractions of eluted samples corresponding to different retention times were collected. Each fraction was then identified with an off-line flow cytometer after cell's labeling with the surface antigen CD34.3 A high enrichment of hematopoietic stem cells in a specific fraction was obtained. Moreover, the potential for the isolation of cells from neoplastic cells present in the same population is also shown. In perspective, the GrFFF system could be employed for the selective fractionation of cell without the use of cell-surface markers that may influence cell differentiation and that are generally absent for immature cells. Flow cytometry can be also used as a control tool on collected samples for direct clinical applications.

HOLLOW-FIBRE FLOW FIELD-FLOW FRACTIONATION WITH TIME-OF-FLIGHT MASS SPECTROMETRY FOR TOP-DOWN PROTEOMICS

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Time-of-Flight Mass Spectrometry (TOF-MS) with soft ion sources, such as Electro-Spray Ionization (ESI) and Matrix-Assisted Laser Desorption Ionization (MALDI), has become a well-accepted and increasingly important technique in top-down proteomics. TOF-MS not only provides the masses and primary sequences of proteins, but also gives information on their higher-order structures and non-covalent interactions. This is due to the ability of soft ionization methods to preserve, at least in part, the native structure of intact proteins and protein complexes in the gas phase, a basic requisite in top-down approaches to protein analysis. Several separation techniques, particularly 2D electrophoresis and reversed-phase High Performance Liquid Chromatography (HPLC), are coupled with TOF-MS for proteome analysis. However, such separation techniques show intrinsic limitations when applied to intact proteins or protein complexes. Flow Field-Flow Fractionation (FlFFF) is a flow-assisted technique particularly suited to the separation of intact proteins. No mechanical or shear stress on sample components is exerted since the fractionation channel does not contain a stationary phase. As mobile phase various carrier liquids can be used to avoid protein degradation. Through FlFFF the protein diffusion coefficients can be directly calculated from retention measurements to obtain information on protein molar mass and conformation.

Since 1974 Hollow-Fibre (HF) membranes were proposed as cylindrical, microcolumn channels for FlFFF. However, the HF-FlFFF performance was only recently improved by the optimisation of the HF-FlFFF fractionator and system design. The HF-FlFFF channel version we have developed is characterised by intrinsic simplicity and low-cost. These features allow for disposable usage of the fractionator, a particularly appealing aspect for analytical bio-applications in which run-to-run repeatability is critical. Together with the low channel volume (*ca.* 100 µl), these features have made HF-FlFFF particularly suited to TOF-MS of whole bacteria and intact proteins.

In this work, we present HF-FlFFF-TOF-MS of intact proteins of pharmaceutical and clinical interest. As an example, rasburicase, an antihyperuricemic drug, was fractionated through HF-FlFFF without affecting its native, tetrametic structure. Rasburicase fractions collected after HF-FlFFF were characterised by TOF-MS. Their enzymatic activity was then measured through a specifically developed, high-sensitive ChemiLuminescence (CL) assay to finally evaluate the activity of the different oligomers.

TETRADENTATE SCHIFF BASE COMPLEXES SYNTHESIS, CHARACTERISATION AND CATALYTIC AND THERMAL PROPERTIES

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In this study, the Schiff base ligands H_2L^1-H_2L^3 and their Cu(II), Co(II), Ni(II), Fe(III), Ru(III) and Mn(II) complexes have been prepared and characterised by spectroscopic and analytical techniques. All complexes have mononuclear and monoelectrolytic nature. When the thermal properties of the complexes are compared, those of Fe(III) have the highest stability. The catalytic properties of the complexes Fe(III), Mn(II) and Ru(III) have been studied using ascorbic acid, 2,6-di-*tert*-butylphenol and 2-*tert*-butylphenol complexes as the substrate. Moreover, the epoxidation properties of the complexes Fe(III), Mn(II) and Ru(III) have been investigated on styrene, 1-pentene and 1-octene.

STRUCTURE OF Cd(II) DITHIOCARBAMATE ATENOLOL COMPLEX

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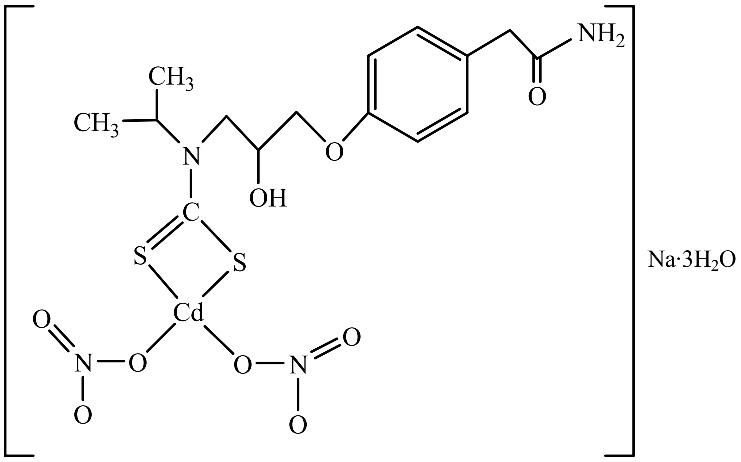
A novel Na salt of dithiocarbamate of the antihypertensive drug atenolol (ATEDTC) and its Cd(II) complex $[Cd(ATEDTC)(NO_3)_2]Na \cdot 3H_2O$ (see the formula below) were prepared and characterised by elemental analysis, Infrared (IR), electronic and magnetic spectroscopy, Atomic Absorption Spectrometry (AAS), Nuclear Magnetic Resonance (NMR) spectroscopy, conductivity measurements and Differential Thermal Analysis - Thermogravimetry (DTA-TG).



DMSO-soluble ATEDTC possesses good chelating ability for various metal ions. Its Cd(II) complex, $[Cd(ATEDTC)(NO_3)_2]Na \cdot 3H_2O$ was an amorphous, intractable solid having polymeric structure. The ligand as well as the complex were found to possess high thermal stability. ATEDTC cyclised on heating to yield a heterocyclic compound which also showed coordinating tendency for Cd ion.

Oral session 2

Novel instrumentation and techniques

Chairperson
A. Roda

DETERMINATION OF ABSOLUTE CONFIGURATION OF CHIRAL DRUGS: NEW CHIROPTICAL SPECTROSCOPIC METHODS

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Enantiomeric forms of chiral drugs exhibit differential pharmacological behavior. Consequently, chiral drugs are increasingly marketed in single enantiomeric form (*e.g.*, the antacid drug S-omeprazole, Nexium).

Drug approval requires the specification of the Absolute Configuration (AC). We have developed new methods for the determination of the ACs of chiral molecules using chiroptical spectroscopies, including Vibrational Circular Dichroism (VCD), Electronic Circular Dichroism (ECD) and Optical Rotatory Dispersion (ORD), in concert with Density Functional Theory (DFT). Examples of the determination of the ACs of chiral organic molecules of pharmaceutical relevance using these methods are presented.

DEVELOPMENT AND COMPARISON OF MICROCHIP OPTICAL SENSING SYSTEMS FOR Ca^{2+} DETERMINATION

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The design and the development of microchip-based fibre optic sensing systems for use in the determination of Ca^{2+} have been carried out. For the determination of Ca^{2+}, two different microchip sensing systems were developed, *i.e.*, an aqueous-aqueous microchip where both the metal ion and the ligand are in aqueous media, the aqueous-solid microchip with the metal ion in aqueous solution and the ligand is on the solid surface. Both microchips have the basic cross-T shape. The main difference between these two microchips is in the channel size. The aqueous-solid microchip has also a cavity, with a diameter of 1000 µm, to host the polymer beads and the channel size of this microchip is 220 µm deep and 470 µm wide, while the aqueous-aqueous microchip has no cavity and its channel size is 255 µm deep and 540 µm wide. Reflectance-absorbance measurements have been used for both systems, although absorbance-based measurements have been a challenge in microchip-based sensing systems due to the micro-scalar dimensions of the channels and the short pathlengths involved.

Cross-linked poly(*p*-chloromethylstyrene) (PCMS) and polyethylenimine (PEI) - attached PCMS beads were prepared by the suspension polymerisation technique for use as a polymeric support. Arsenazo III (1,8-dihydroxynaphthalene-3,6-disulfonic acid-2,7-bis [(azo-2)- phenylarsonic acid]) has been used as a Ca-binding reagent due to its high affinity for the free Ca^{2+} in the sample. Optimum conditions for the immobilization of this ligand onto polymeric support were also investigated. The maximum amount of electrostatically immobilized Arsenazo III onto PEI-attached PCMS beads was found to be 373.71 mg g^{-1} polymer.

The operational parameters of the sensing systems, such as pH, flow rate, applied electric field, response time and the regeneration conditions have been optimised. In addition, the reproducibility of the measurements, Relative Standard Deviation (RSD) of the methods and the Limits of Detection (LODs) of the analysis have been determined for both measurement systems.

RECENT ADVANCES ON BIOSENSORS BASED ON ENZYME INHIBITION

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Analytical technology based on sensors is an extremely broad field which impacts on all major industrial sectors such as the pharmaceutical, healthcare, food, agriculture, industrial and environmental sectors. Applications of biosensors based on enzyme inhibition are still limited since these sensor technologies are not usually able to discriminate various toxic compounds in the same sample. However, biosensors can be used as alarm systems; they would provide either quantification of one contaminant when the analyte is present alone or give an indication of the total contamination of the particular samples. They could also be used as a complementary technique to classical methods. On the other hand, a particular advantage of these biosensing systems is that they offer the possibility of analysis in both batch and flow mode, allowing these sensors to be used for analysis of a large number of samples in a reasonable time interval. These methods can be recommended also for a single use with screen-printed electrodes to avoid problems related to electrode fouling which generally involves a chemical or electrochemical deactivation of the working electrode surface. Screen-Printed Electrodes (SPEs) offer several additional advantages including low cost, simple handling, mass production and suitability for instrument miniaturisation for *in loco* analysis. Also, the disposable use of these sensors avoids the need of reactivation.

This lecture summarises the research carried out during the last five years on biosensors that are based on enzyme inhibition for the determination of pollutants and toxic compounds in a wide range of food and environmental samples. The general approaches to enzyme immobilization, enzyme catalysis and inhibition mechanism are also highlighted.

Work on biosensor inhibition developed in the Authors' respective laboratories in Morocco and in Italy is illustrated. Results of invertase inhibition by heavy metals and cholinesterase inhibition by pesticides are discussed in detail. These biosensors represent cost-effective analytical procedures that can be easily employed in third world laboratories.

SOLAR UVB RADIATION AND VITAMIN D SYNTHESIS IN BIOSPHERE: NEW METHOD OF AN *IN SITU* MONITORING

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Although UV radiation is classified as non-ionizing radiation, significant biological activity of the UVB radiation (280-315 nm) is well known. Excessive UV exposure is generally associated with acute and chronic health effects, even if at proper doses UV radiation is beneficial, in particular because of the generation of vitamin D3 in skin. To date endogenous synthesis of vitamin D is the most well known and well documented beneficial effect of solar UVB radiation. All higher vertebrates as well as most plants (including phyto- and zooplankton), avian species and aquatic mammals, have an endogenous mechanism involving solar UVB radiation which affects the synthesis of vitamin D from its precursor.

Sunlight has long been recognised as the main source of vitamin D3 for humans. Vitamin D's main function is to maintain normal levels of Ca and P in the blood. As one of the body's many control systems, vitamin D is a critical hormone that helps regulate the health of more than 30 different tissues, from the brain to the prostate. It plays a role in regulating cell growth, the immune system and blood pressure. Dramatic seasonal and latitudinal effects on the vitamin D synthetic capacity of sunlight correlate with recent epidemiological findings: the chronic diseases associated with vitamin D deficiency are 25 to 50% more frequent in northern climates than among people living closer to equator. Besides, stratospheric ozone depletion gives rise to the increase of solar UVB, whereas air pollutants and aerosols hinder penetration of essential solar UVB radiation into the biosphere.

These facts testify that an *in situ* control of solar UV radiation with respect to its ability to initiate vitamin D synthesis demands particular care. However, at the moment no instrument is produced to measure *in situ* the vitamin D synthetic capacity of sunlight (or artificial UV sources). Commonly used broad-band radiometers with spectral sensitivity corresponding to the Commission Internationale de l'Éclairage (CIE) erythema action spectrum are unable to measure correctly the vitamin D synthetic capacity of sunlight in view of significant differences in the action spectra.

Two methods have been developed for direct evaluation of the vitamin D synthetic capacity of sunlight (and artificial UV sources). The first one uses an *in vitro* model of vitamin D3 synthesis (ethanol solution of 7-dehydrocholesterol, 7-DHC) and concentration of previtamin D3 accumulated during a UV exposure is determined using especially designed spectrophotometric analysis. The second method utilises photoisomerization of provitamin D in nematic liquid crystalline (LC) matrix and visual estimation of accumulated previtamin D becomes possible due to special design of an LC cell. This user-friendly method is appropriate for personal UV dosimetry and may have wide application in tanning saloons, in clinical dermatology and in UV therapy. It is vital to note that, whereas there are both beneficial and detrimental UVB effects, it is the increase in the detrimental effects, associated with ozone depletion, which attracts now most attention. Concerns range from human health (increase in the incidence of skin cancer), to agriculture (reduction in crop yields) and to aquatic ecosystems (changes in the world's oceans).

To raise public awareness and to provide information about potential risks of solar UV exposure, the use of a simple universally applicable solar UV index has been recommended by the World Health Organization (WHO) in 1994. This UV index accounts for minimum erythema dose (1 MED ≈ 200 J/m^2) and is calculated from the spectroradiometry data using the CIE erythema action spectrum. *In situ* monitoring of the vitamin D synthetic capacity of sunlight paves the way to the introduction of new UV D index that will provide public information (and education) about the lowest vitally important dose of solar exposure for adequate endogenous synthesis of vitamin D considering substantial disease burden associated with both excessive exposure to UV radiation and inadequate exposure to sunlight. Ideally, global UV mapping as well as annual and daily UV forecasts should consider both erythemal and "antirachitic" solar UV indices.

DIFFUSIVE SAMPLERS:
A TOOL FOR PRELIMINARY ASSESSMENT.
VALIDATION OF THE ANALYST® SAMPLER

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Data quality objectives specified for different kinds of measurement methods, including diffusive sampling, are set by the three recently published EU Air Quality Daughter Directives (covering the gaseous species SO_2, NO_x, NO_2, benzene and O_3). Diffusive sampling is a simple and low-cost sampling method to collect gases and vapours alternative to traditional methods. The ANALYST® diffusive sampler is a modification of the open-tube design which employs a filter impregnated with reagents able to trap the pollutant. Type-approval test procedures have been developed for diffusive sampling by the Comité European de Normalisation (CEN). This procedure requires all contributions to the total measurement uncertainty of the method arising from different performance characteristics (*e.g.*, linearity and cross-interferences) to be identified and tested to ensure that the combined expanded measurement uncertainty (calculated from all possible components) meets the data quality objectives. Maximum measurement uncertainties allowed for by the results obtained using the selected method are also defined. This is a rather complex and time-consuming procedure that goes along with a rigorous protocol for determining sampler performance of diffusive samplers.

The validation procedure adopted for the ANALYST® sampler reported in this presentation is based on four aspects:

1) laboratory tests concerning linearity of response (*i.e.*, the extent to which response is directly proportional to input. A sorbent that does not linearly respond to a changing input concentration – *e.g.*, DPE in the case of ozone - should be not further evaluated);
2) laboratory tests concerning sorption efficiency, capacity and humidity on the sorbent and of wind speed on the device as such;
3) study of whether the samplers exposed for extended periods (weeks or months) give the same integrated response as a series of short-term samplers run side-by-side. This gives information on both capacity and interference of other pollutants;
4) field validation using a second method as reference according to ISO/FDIS 13752.

It was found that the ANALYST® sampler is capable of reliable measurements of the mentioned pollutants (and others such as HNO_2, HNO_3 and H_2S) at common levels found in polluted atmosphere in urban, rural and remote environments. It provides average concentration levels over one month and even more, well within the expanded uncertainty under field conditions requested by the European Directives. The results obtained are reported and discussed. The relative importance of laboratory *vs.* field performance experiments and the methodology of validation is also addressed.

RELIABLE QUANTITATIVE THIN-LAYER CHROMATOGRAPHY

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Quantitative Thin-Layer Chromatography (QTLC) measured by direct photometric scanning is used since more than fifty years. Nevertheless, QTLC is not so popular as it should be according to metrological expectations, although it is a very reliable analytical technique. It is a flexible method with many not precisely controllable parameters, which on the one hand contribute to large dispersion of measurements, whereas, on the other hand, make this method insensitive to systematic errors. QTLC has a unique advantage in it that processes a large number of samples in one run. This specific situation makes it one of the most informative microanalytical techniques. QTLC collects data from different samples at one time, while with column separations data can be compared only in the case of robust measuring conditions.

In modern QTLC accuracy is achieved with the use of automated and standardized operations. The improvement in reproducibility, simplicity and speed is obtained with automatic sample manipulation, controlled chromatographic procedures and the use of densitometry or video scanning. Video techniques are not so sensitive as densitometry, but the response from a whole plate is very rapid and scanning parameters are easily adapted to plate conditions. In practice, many users and even instrument manufacturers think that both data acquisition methods are similar and that there is only a slight difference according to the type of sensors. Experimental work showed basic theoretical and practical differences between these data acquisition modes. These differences might have an important influence on the validity of the results of each of these two scanning techniques.

The drying stage is the major source of error. During drying process mobile phase evaporates from the upper part of the plate. Solvents inside a plate travel through stationary phase to the surface and molecules of separated components inside a layer move up or down. Experimental evidence shows that there is a strong dependence between the inner position of spots and the measured signal. This uncontrolled secondary chromatographic process is the main reason for poor precision in QTLC. With up to 10% RSD it is by far the biggest source of uncertainty. In order to reduce this influence it is necessary to control the speed of the evaporation. The influence of inhomogeneous distribution is further reduced by increasing the number of applications of the same sample or with the application in form of bands and properly selected drying procedure.

A new type of dryer for QTLC plates has been developed and patented. Due to the laminar and temperature-controlled flow of air or inert gas, the in-depth distribution of separated compounds is controlled and standardized. This device is now routinely used in the Authors' laboratory and also in laboratories of the pharmaceutical sector.

ZINC (II) PVC-BASED MEMBRANE SENSOR USING 3-[(2- FURYLMETHYLENE)AMINO]-2-THIOXO-1,3-THIAZOLIDIN-4-ONE

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The 3-[(2-furylmethylene)amino]-2-thioxo-1, 3-thiazolidin-4-one (FTT) was used as an excellent ionophore for developing a Zn(II) PVC-based membrane sensor. The best performance was obtained with a membrane composition of 30% poly(vinyl chloride), 62% nitrobenzene (NB), 3% FTT and 5% Na tetraphenyl borate (TBP). This sensor shows very good selectivity and sensitivity towards Zn ions over a wide variety of cations, including alkali, alkaline earth, transition and heavy metal ions. The sensor revealed a great enhancement in selectivity coefficients for Zn ions in comparison with the previously reported Zn sensors. Theoretical studies also show the selective interaction of TFF and Zn ions. The proposed sensor exhibits a Nernstian behavior (with slope of 29.3 ± 0.3 mV per decade) over a wide concentration range (1.0×10^{-6} - 1.0×10^{-2}) with a detection limit of 8.5×10^{-7} *M* (52 ng ml^{-1}). It shows a relatively fast response time over the whole concentration ranges (< 20 s) and can be used for at least 10 weeks in the pH range of 3.0-7.0. The proposed sensor was successfully used for the direct determination of Zn ions in waste water of industrial Zn electroplating and also as an indicator electrode in titration with EDTA.

IN-DEPTH PROFILE QUANTIFICATION OF THIN LAYERS ON NON-CONDUCTING GLASSES BY RADIO FREQUENCY GLOW DISCHARGE COUPLED TO ATOMIC EMISSION SPECTROMETRY

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At present, the radiofrequency (rf) - Glow Discharge (GD) coupled to Atomic Emission Spectrometry (AES) is considered as a useful technique for the analysis of materials, both for homogeneous samples and coatings. Thin films and coatings are used in a great number of applications in different fields of technology, going from microelectronics and optics to protective coatings against various influences. Most of the physical properties of the thin films depend on their analytical characteristics, such as the chemical composition and structure. Rf-GD-AES devices have been applied for depth profiling analysis of many different types of samples, athough it should be mentioned that for the vast majority of these applications samples should be conductive.

The analysis of thin conductive or insulating layers on glasses is a field of high interest in research and also for quality control in the industrial field (*e.g.*, antireflective coatings and coatings for thermal isolation on glass are commonly employed nowadays). Rf-GD-AES was demonstrated to be able to perform elemental depth profiling in non-conducting materials at a high spatial resolution down to the nano-scale, but quantification requires further work on normalization strategies to compensate for variations in elemental response factors between the different materials.

The constant emission yield concept has been widely employed for multimatrix calibrations addressed to the analysis of homogeneous conducting and non-conducting materials with GD-AES. However, the use of emission yields has not been investigated so far for the quantification of coated insulator materials. The capabilities of rf-GD-AES for the quantification of different kinds of thin films (less than 100 nm thickness) on several non-conducting glass samples (with thickness in the range between 1.8 and 4.8 mm) has been investigated in detail by resorting to the constant emission yield concept, using for calibration a set of silicate glasses with a wide range of elemental concentrations and several conducting standard reference materials in different matrices.

PREPARATION OF ULTRATHIN FILMS OF POLYANILINE BY ELECTROGENERATED OXIDANT ON GOLD SURFACE

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The properties of conducting polymers are attracting more and more interest due to their unique applications. Switching of conducting polymers between the conducting and non-conducting mode can be effectively exploited only for very thin films of high integrity. The ultrathin films of conducting polymers have properties in improving the response time for sensors. The quality and performance of the polymers depend mainly on the adopted synthesis method. The control of the structure and morphology of the films is thus essential when envisioning practical applications. Therefore, for the development of new methods, besides classical chemical and electrochemical synthesis, it is necessary to achieve the needed special features for conducting polymers. However, it is difficult to obtain a very thin and even film of conducting polymers with thickness at the controllable molecular level by conventional anodic polymerisation.

In this study, aniline monomer was oxidised to polyaniline (PANI) by electrogenerated oxidant produced by the reduction of oxygen. Polymerisation was carried out at the reduction potential of oxygen and direct anodic oxidation of aniline was thus avoided. The properties of the prepared films were examined by electrochemical and STM techniques. It was ascertained that switching between conducting and non-conducting forms was possible. The relationship between the peak current obtained from ultrathin polyaniline electrodes and the density of polyaniline on gold surface was also investigated.

SPECIATION OF Cr(III) AND Cr(VI) BY THE COMBINATION OF SOLID PHASE EXTRACTION AND SPECTROPHOTOMETRY

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In environmental samples, Cr is one of the most frequent pollutants. Chromium is a major water pollutant, usually as a result of some industrial pollution due to tanning factories, steel works, industrial electroplating, wood preservation and artificial fertilizers. Chromium exists in the environment at the Cr(III) and Cr(VI) oxidation states. The chemical properties of these species are different. Chromium (III) is considered to be an essential nutrient for the maintance of normal glucose tolerance, whereas Cr(VI) can have acute and chronic toxic, including carcinogenic effects. For these reasons, the determination of Cr species in environmental samples is very important.

In the present study, a simple and sensitive method for the speciation, separation and preconcentration of Cr(VI) and Cr(III) in environmental samples was developed. Chromium (III) has been separated from Cr(VI) as Cr(III)-diphenylcarbazone complex on Ambersorb 563 resin. The adsorbed complex was eluted from the column with acetone and determined by UV-Vis spectrophotometry at 540 nm. The effects of analytical parameters such as reagent concentrations, type of elution solution, sample volume, amount of resin and foreign ions were investigated. The procedure described is successfully applied to the Cr speciation in natural waters, sediments and soils.

A GAS CHROMATOGRAPHY MASS SPECTROMETRY ANALYTICAL PROCEDURE FOR THE DETERMINATION OF LIPIDS, WAXES, RESINS, PITCH, TAR AND PROTEINS IN MICRO-SAMPLES FROM PAINTED WORKS OF ART

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Samples from painted works of art are generally characterised by the concomitant presence of several different organic materials together with pigments and other inorganic components (support, metal leaf). The organic content is commonly quite low in respect to the inorganic one and due to the artistic value the sampling is executed minimising the damage to the artwork. Hence, paint samples are very limited in size (frequently less than 100 μg). All these reasons represent a challenge in the characterisation of lipids, waxes, proteins and resinous materials that simultaneously can be present in the same sample.

A novel analytical procedure is described for the identification of the above mentioned materials in the same paint micro-samples. It is based on a sample pre-treatment capable to separate the various organic components into different fractions, that are properly treated and derivatised and then analysed by means of Gas Chromatography-Mass Spectrometry (GC-MS).

This method achieves the quantitative determination of the amino acid profile, permitting to distinguish between different proteinaceous materials (egg, collagen, casein) by means of a statistical data treatment based on principal component analysis. The identification of lipids (linseed oil, poppy seed oil, walnut oil and egg), plant resins (pinaceae resins, sandarac, mastic and dammar), animal resins (shellac), tar or pitches and natural waxes (beeswax, carnauba wax) is performed on the basis of the determination of fatty acid, alcohol and hydrocarbons profiles and of significant terpenic molecular markers.

The application of the analytical procedure to samples from easel paintings of the XVI and XVII century and from archaeological objects is also reported and the identification of the organic components is discussed.

A NOVEL RAPID METHOD FOR THE IDENTIFICATION OF *THUNNUS ALBACARES* (YELLOWFIN TUNA), *THUNNUS OBESUS* (BIGEYE TUNA) AND *KATSUWONUS PELAMIS* (SKIPJACK) DNA FROM FRESH, FROZEN AND CANNED TUNA TO FIGHT COMMERCIAL FRAUDS

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The tuna family includes several closely related members such as yellowfin, bigeye and skipjack with different commercial values. These can be sold as fresh fish or processed food such as canned and frozen products. In particular, the identification of fish, necessary to prevent fraudulent substitutions in the fish market, can be difficult when morphological features, such as fins, head and skin are removed during processing. Since DNA is more thermostable than proteins, DNA-based methods in food forensics represent an attractive approach to fight fraudulent mislabelling of processed food and to protect the consumer.

A direct method for discriminating between yellowfin tuna, bigeye tuna and skipjack DNA was developed based on the base differences identified as a result of the sequence alignments. Sequences in the mitochondrial DNA cytochrome b gene (mtDNA cyt b) have been used for species identification. The use of a mitochondrial gene may present advantages over nuclear DNA because of its relatively high abundance, allowing for an easier detection and, since mitochondria remain intact after processing, loss of DNA is avoided. As with all mitochondrial DNA analysis, the possibility of intraspecific variability was first considered. DNA was extracted from frozen, cooked and canned samples. Sequencing was undertaken using ten samples from each species, a 577 base pair (bp) amplicon was generated by Polymerase Chain Reaction (PCR) and sequenced. The three species were analysed to determine the intraspecific variation and the positions with diagnostic value. Polymorphic sites between the species that did not present intraspecific variation were given a diagnostic value. According to these diagnostic nucleotides a set of species-specific primers were designed to obtain a species-specific PCR amplified of 246 bp fragments for yellowfin, 262 for bigeye and 113 for skipjack.

This method relies on species-specific amplification products being generated only in the presence of the correct template nucleic acid and the species of origin of the DNA is indicated by the distinctive size of the PCR product. A simple agarose gel electrophoretic analysis of the amplicons proved to be capable of leading to unambiguous identification of the three species. All the analysed samples were correctly assigned to the corresponding species. The sequencing of PCR products was used as confirmatory analysis. This simple determination method is faster than any other method described so far for the identification of tuna commercial products with good accuracy, low cost and possible automation for large-scale analysis. The method thus represents a valuable tool to determine and confirm tuna identity with potential applications for routine works such as surveys of quality and labelling of commercial tuna preparations.

Poster session 1

Fundamentals, novel instrumentation and techniques

Chairperson
P.J. Stephens

THE PREPARATION OF HIGH CAPACITY ADSORBING SILICA GEL

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Silica gel consists of silicic acid hydrate in a dry aggregate state (kserogel) having porosity and surface area as high as possible to improve the adsorption power. The interest for silica gel is due to its important adsorbing properties, basically high capacity and selectivity, ability to be regenerated for several times without loss of efficiency and high stability when in the state of powder or grains.

This work describes three different preparation methods to obtain a product with the best adsorbing characteristics as tested on three target compounds assumed as markers, namely, benzene, water and methylene-blue. The three preparation methods considered are based on Na_2SiO_3 as raw material treated alternatively with bubbling CO_2, hydrolysing solution of NH_4Cl + NaCl and sulphuric acid solution, each one of these methods being addressed to ensure that silica gel has the qualities requested by the market. Particular attention was paid to the form of raw material and to the experimental conditions (particularly of precipitation, washing, drying, gelifying) as they determine the alternative formation of micro- and macro-porous gel. The comparison beetween the known adsorption values and the ones experimentally determined on the samples confirm the good performance of the proposed methods, the third one of which ($Na_2SiO_3 + H_2SO_4$) resulted in the highest adsorbing properties.

THE INFLUENCE OF HYDROGEN ADDITIONS ON DIRECT CURRENT AND RADIOFREQUENCY ARGON GLOW DISCHARGE - MASS SPECTROMETRY

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Glow Discharges (GDs) are used in a wide variety of fields in modern science and technology. In analytical chemistry, GDs are employed as atomisation/excitation/ionization sources and the main applications are addressed to the analysis of bulk materials, coatings and surfaces. The GD is generated in a low pressure (a few torr) gaseous medium (usually Ar as inert atmosphere). The discharge can be generated directly either by direct current (dc) used for conducting materials or by radiofrequency (rf) which can be used also for insulating materials. The usual characteristics of a GD plasma are drastically varied by several discharge parameters, such as voltage, electrical current, power (especially with rf power supplies) and gas pressure. Moreover, the plasma gas plays an important role both in the sample sputtering rates and in the excitation/ionization of sample atoms. In recent years, special attention has been devoted to investigate the features of GD sources using a wide variety of plasma gases.

Nowadays, GD analytical detection is mostly carried out either by Atomic Emission Spectrometry (AES) or Mass Spectrometry (MS). The use of GDs coupled to MS is increasing because of the recognised advantages in terms of sensitivity, spectral simplicity, isotopic information *etc*. In particular, the use of Time of Flight (TOF) mass analysers raises particular interest because of the capability to collect the complete mass spectra with the same precision, sensitivity and resolution regardless of the total number of isotopes being measured.

In a previous work a preliminary study was carried out about the effect of Ar-H_2 mixtures on dc-GD-TOF-MS while keeping constant the electrical current and voltage and some interesting features were observed. In this communication, a thorough and comparative study is presented about the effect of these plasma gas mixtures for dc (fixed pressure and voltage) and rf (working at constant pressure and forward power) GDs coupled to TOF-MS in terms of sputtering rates, ion signals, ion yields and depth resolution for the analysis of thin films.

CATALYTIC ACTIVITY AND ELECTROCHEMICAL AND THERMAL STUDIES OF THE MIXED LIGAND COMPLEXES

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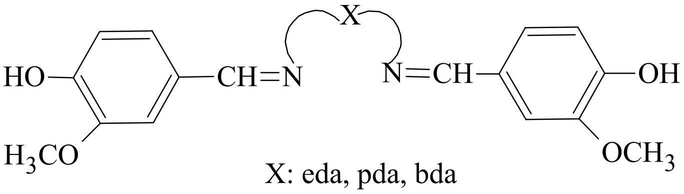
In this study, the Schiff base ligand (H_2L) and its Cu(II), Co(II), Ni(II), Fe(III) and Ru(III) complexes have been prepared and characterised by various spectroscopic and analytical methods. The H_2L has been obtained from reaction of the vaniline and various diamines (ethylenediamine, eda; propylenediamine, pda; butylenediamine, bda) in the ethanol media. The general structure of the Schiff base is given below.



As the ligand has two donor atoms, its mixed ligand complexes have been synthesised using a 1: 1: 1 molar ratio (H_2L: M: X, with X *o*-phenantroline, biquinoline and dipyridyl). Cylic voltammetry of the Cu(II), Co(II) and Fe(III) complexes was carried out. The catalytic properties of the complexes have been studied using the 2,6-di-*tert*-butylphenol and ascorbic acid. Moreover, the epoxidation properties of the Fe(III) and Ru(III) complexes have been studied for styrene, 1-pentene and 1-octene.

TRANSITION METAL COMPLEXES OF SOME ONNO TYPE SCHIFF BASE LIGANDS: THEIR SYNTHESIS AND THERMAL AND ELECTROCHEMICAL PROPERTIES

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In this study, first the amine compounds H3A and H3B were synthesized as the starting compounds and then the ligands H3L^1-H3L^3 were prepared with H2L^4 through the reaction of H3A and H3B with 4-methyl-2,6-di-formlyphenol and 4-*tert*-butyl-2,6-di-formylphenol in ethanol media. All compounds have been characterised by spectroscopic and analytical methods. In the electronic spectra of the some complexes of the Schiff base ligands, the 480-410 nm band has been attributed to the charge transfer band. While the ligand H_2L^4 has four potential donor atoms, other ligands have many more. In order to investigate the keto-enol tautomeric equilibria of the ligands, the spectra of the ligands have been recorded in polar and non-polar solvents. While the ligands prefer the enol-imine form in polar solvents, such as EtOH, MeOH and $CHCl_3$, in non-polar solvents (hexane, heptane and toluene), the ligands convert to keto-amine forms. Antimicrobial activities of the ligands have been investigated against the bacteria and fungi and found that some of them have high activity. In general, many of the ligands show lowest activity against the *S. cerevisiae* and *E. coli*. The antimicrobial activities of the Cd(II) complexes are lower than those of the ligands. The complexation reduces the antimicrobial activity. Electrochemical properties of the Ni(II) complexes have been also studied.

SYNTHESIS, CATALYTIC ACTIVITY AND IMINE BOND ACTIVATION OF SOME Cu(II) SCHIFF BASE COMPLEXES

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In this study, the Schiff base ligands HL^1-HL^6 and their Cu(II) complexes have been obtained from the reaction of the ligands with Cu(II) salt in EtOH. The structure proposed for the Schiff base ligands HL^1-HL^6 is given below. All compounds have been characterised by spectroscopic and analytical methods. The catalytic activity studies of the complexes have been done on the ascorbic acid and the sterically hindered phenol 2,6-di-*tert*-butylphenol. Moreover, the electrochemical behaviour of several Cu(II) complexes with Schiff base ligands have been investigated in aprotic solvents by cyclic voltammetry. The azomethine bond of the Schiff bases in HL^1-HL^6 after treatment with H_2O_2 undergoes oxidative conversion to a bis(salisylaldiminato) Cu(II) or (2,6-formylphenolato) Cu(II) hydrate species through the formation of an amido intermediate as evidenced from solution infrared spectral studies.

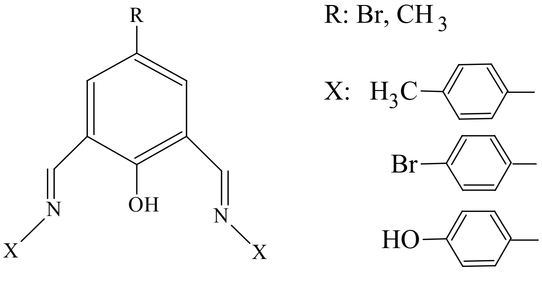

Figure. Proposed structure of the Schiff base ligands HL^1-HL^6

STRUCTURAL CHARACTERISATION OF FISETIN-Cu(II) COMPLEX

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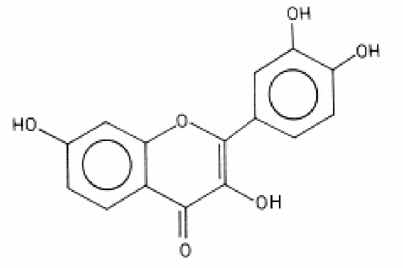
Fisetin (3,3′,4′,7-tetrahydroxyflavone) (see structure below) is a naturally occurring polyhydroxyflavone which has raised significant spectroscopic interest for its dual emission behaviour and excellent lasing properties. It has also gained much attention from a biological viewpoint for its inhibitory action against protein kinase, a signal transducing enzyme and HIV-1 proteinase, a virally encoded protein which is pivotal to the maturation and activity of AIDS virus and a viable target for anti-HIV therapy.



In this study, the synthesis and spectral (UV–Vis, ^{1}H-NMR, IR) properties as well as thermal behaviours of Cu(II) complex with the flavonoid fisetin (L) is presented. The complex may be formulated as $[Cu_2(L)(H_2O)_4]Cl$. The structure, stability and molar absorptivity of the complex formed between $CuCl_2{\cdot}H_2O$ and 3,3′,4′,7-tetrahydroxyflavone in methanol were investigated using UV-Vis spectroscopy. The molar ratio method and Job's method of continuous variation were applied to ascertain the stoichiometric composition of the complex in methanol at constant ionic strength. A 1:2 (L:M) complex was assumed by this method. The molar absorptivity and stability constant of the complex were determined using a simple and accurate procedure that requires solutions having the ligand and metal ion at the stoichiometric proportion. The high stability constant demonstrates that the complexation reaction is total.

DINUCLEAR AND MONONUCLEAR SCHIFF BASE METAL COMPLEXES: STUDIES OF THEIR CATALYTIC AND ANTIMICROBIAL ACTIVITY PROPERTIES

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The reaction at the 1:1 or 1:2 L:M molar ratio of the ligand HL and Cu(II), Co(II), Ni(II), Pb(II) and Zn(II) ions resulted in the formation of the mononuclear [with the exception of Pb(II)] and homodinuclear metal complexes. Moreover, the mononuclear complexes Fe(III) and Ru(III) have also been synthesized. The heterodinuclear complexes $[PbM(L)Cl]_2Cl$ (M: Cu, Co, Ni or Zn) have been obtained from the reaction of the complex $[Pb_2(L)AcO]_2AcO$ and Cu(II), Co(II), Ni(II) and Zn(II) transition metal salts. The ligand and its complexes have been characterised on the basis of analytical and spectroscopic data. All complexes have electrolytic nature. Homo- and heterodinuclear Cu(II) and Co(II) complexes have a subnormal magnetic moment. The Fe(III) and Ru(III) complexes are found to be a good catalyst for the oxidative C-C coupling of hindered phenols to diphenoquinones. The ligand HL shows the keto-enol tautomeric forms in polar and apolar organic solvents. ^{1}H-(^{13C})- NMRspectra of the diamagnetic $[Ni_2(L)Cl]_2Cl$ and $[Zn_2(L)Cl]_2Cl$ complexes have been also recorded. In addition, the antimicrobial activity properties of the ligand and its homo- and heterodinuclear complexes have been studied against bacteria and fungi. Likewise, results have been compared in the case of antibacterial and fungi drugs. Thermal analysis of all complexes was carried out. The TGA curves show that the decomposition takes place in three steps.

PREPARATION OF HIGH ANTIMICROBIAL ACTIVITY OF 1-[(1-METHYLETHYL)AMINO]-3-(1-NAPHTHALENEOXY)-2-PROPANOL-SODIUMDITHIOCARBAMATE-Zn(II) COMPLEX

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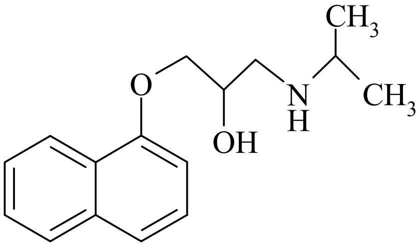
Propranolol (1-[(1-methylethyl)amino]-3-(1-naphthaleneoxy)-2-propanol) (see structure below) is a drug often used in the treatment of hypertension, angina and thyrotoxicosis which is almost completely metabolised in man. The major metabolites identified in plasma and urine include conjugates of this drug.



The propranolol-Na dithiocarbamate-Zn(II) complex was prepared through reaction of CS_2 with NaOH in methanol. The complex was characterised by UV, FT-IR, ^{1}H-NMR, and elemental analysis. Sulphur atoms coordinated to Zn ions and were the major electron donors. The antimicrobial activitiy of complex was tested using the disc diffusion method against some bacterias and yests such as *B. Megaterium*, *B. Brevis*, *Y. Enterecolitica*, *M. Luteus*, *P. Aeroginosa* and *E. Feacelis*. The complex showed a wide spectrum (inhibition zone, 19-25 mm) of antimicrobial activities.

INVESTIGATION OF ADSORPTION ISOTHERMS USED FOR WOOL OF WALNUT (*JUGLANS REGIA L.*)

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The assessment of dyeing processes is based on the adsorption equilibrium of the dye distributed between dyestuff solutions and the fibres to be dyed. The dyeing distribution is expressed by isotherms drawn as the concentration of the dye adsorbed on the fibres (C_a) against the equilibrium concentration of the dye in solution (C_d). These isoterms are classified as Nernst, Freundlich and Langmuir isoterms. Langmuir and Freundlich isotherms were originally derived for the adsorption of gases by solids, the Nernst isotherm is common in dyeing processes. In this isotherm type, the distribution of the substance to be adsorbed between the solid and solvent phases is expressed by a proportionality constant. There is a constant ratio between the adsorbed and aqueous concentrations of the adsorbate with the slope of the isotherm equal to K. If the same dyestuff adsorbed on various adsorbents gives Nernst's type isotherms, there exists a direct relationship between K and the extent of dyestuff adsorption. The Nernst equation is then $C_a = KC_d$.

In this work, adsorption isotherms were obtained by processing wool with dyestuff solutions of different concentrations extracted from walnut with water. These isotherms follow the Nernst equation; the Nernst constant was determined and interpreted.

INVESTIGATION OF COLD PLASMA SECTOR FIELD INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY FOR THE DIRECT DETERMINATION OF ULTRATRACE ELEMENTS IN COMPLEX SAMPLE MATRICES

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The need to analyse pg g^{-1} levels of elements in complex sample matrices occurs in all areas of application, such as seawater, brines and bitterns from the environmental and geochemical fields; blood, serum and urine from clinical applications; concentrated acids, organic solvents and photoresists from the semiconductor industry.

Such matrices are difficult to analyse with quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Direct analysis of difficult samples offers several advantages over matrix removal procedures, including minimising the risk of contamination, avoiding the loss of the analyte during sample preparation and increasing the speed of the analytical procedure. The problems that must be addressed in the direct analysis of complex matrices include spectral interferences caused by the matrix, signal suppression and deposition of matrix on the cones.

The use of a high resolution ICP-MS (Thermo Electron Element 2) allows difficult matrices to be directly analysed because interferences can be mass resolved from the analytes of interest and because the high sensitivity of sector field instruments allows high dilution factors to be used without sacrificing detection limits.

For ultra trace element determinations of Fe, K, Li and Na, measurements can be performed using cold plasma to decrease Background Equivalent Concentration (BEC) values for these elements. High resolution is used to eliminate the new spectral interferences, which arise in complex matrices under cold plasma conditions.

OVERCOMING MATRIX OXIDES WITH THE AGILENT 7500 CE. ANOTHER STEP CLOSER TO INTERFERENCE-FREE INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY ANALYSIS

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Agilent's unique Octopole Reaction System (ORS), operating in the He collision mode, can virtually eliminate matrix oxides from the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) spectrum, even in high matrix sample types, without the compromises associated with the use of complex desolvation accessories. The CeO peak at mass 156 has been reduced to near-background levels. The actual ratio of CeO/Ce is 0.07 %, the lowest ever reported for an ICP-MS operating with a conventional concentric nebulizer and without the use of aerosol desolvation. The same He mode conditions can be applied to the removal of any polyatomic ion, whether known and constant or unidentified and variable, on any analyte. This unlocks previously interfered elements in many high matrix sample types and enables the accurate measurement of high mass Rare Earth Elements (REEs) in natural samples. The 7500ce is revolutionizing ICP-MS analysis, finally allowing analysts to achieve the full potential of the technique. The 7500ce combines the power of high resolution ICP-MS with the flexibility, ease of use and low cost of quadrupole ICP-MS.

THE 7500 OCTOPOLE REACTION SYSTEM INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY. HELIUM COLLISION MODE REMOVES ALL MATRIX INTERFERENCES

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Spectra were acquired in an extremely complex sample matrix containing 1% HNO_3, 1% HCl, 1% Butanol, 1% H_2SO_4 and 100 μg g^{-1} each of Ca and Na. No background subtraction or other spectral correction were used.

All matrix-derived polyatomic backgrounds are removed and only trace Ar_2 at m/z 80 remains. The remaining elemental peaks are due to pg g^{-1} level contamination in the matrix, as confirmed by correct isotopic template fit. The He collision mode thus provides: *i*) essentially interference-free analysis in any matrix; *ii*) no need for knowledge of sample matrix; *iii*) highest data integrity in unknown matrices.

DEVELOPMENT OF HPLC-MS METHODS TO INVESTIGATE THE SPECIATION OF Se

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The nutritional importance of Se is the object of recent discussions. On the one hand, Se has been recognised as essential to life since, *e.g.*, it plays a protective role against the toxicity of heavy metals whereas several diseases can be ascribed to Se deficiency. On the other hand, Se can become toxic when ingested at high amounts, as for example in the uncontrolled use of nutritional Se supplements. It is not easy to evaluate what the correct assumption of this element should be, as the human organism may absorb Se in several ways, depending on the kind of food and on the different inorganic and organic species and oxidation states under which Se can be present. Aminoacidic forms are generally more bioavailable and potentially less toxic than the inorganic ones, but possible reactions involving inorganic and organic species can take place also *in vivo*. Thus, *e.g.*, selenide is the precursor of Se aminoacids species.

This study describes the development of suitable methods for the identification and determination of the most relevant inorganic and organic species in food, nutritional supplements and biological samples. High Performance Liquid Chromatography (HPLC) methods have been developed for the following species: metylselenic acid (CH_3SeO_2H), selenate (SeO_4^{2-}), selenocyanate ($SeCN^-$), selenite (SeO_3^{2-}), seleno-L-methionine ($C_5H_{11}O_2NSe$), seleno-L-cystine ($C_6H_{12}O_4N_2Se_2$), phenyl-L-selenocysteine ($C_9H_{11}O_2NSe$) and methyl-L-selenocysteine ($C_4H_9O_2NSe$). Since UV-Vis detection could be employed only for phenyl-L-selenocysteine, Mass Spectrometry (MS) was resorted to for the detection of all analytes.

The entire identification and separation procedure also involves the development and optimisation of the method of extraction of the analytes from the matrix and the statistical evaluation of the mean extraction recovery. The development, optimisation and validation of the HPLC-MS method to be used also in complex matrices for the identification and determination of the species of interest, are based on the information collected beforehand through the following steps; *i*) characterisation in Electro-Spray Ionization (ESI) mass spectroscopy; *ii*) MS fragmentation studies in ion trap analyser; and *iii*) flow injection analysis to estimate the interaction between analyte and mobile phase.

CHROMIUM SPECIATION ANALYSIS BY SEPARATION OF Cr(III) FROM Cr(VI) ON MALEATE- AND SHELLAC-DERIVATISED XAD RESINS

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Chromium exist in aqueous systems in basically two oxidation states, namely Cr(III) and Cr(VI), whose toxicities are significantly different. Although Cr(III) is an essential trace element for living organisms, Cr(VI) is toxic and carcinogenic. This study was based on two different sorbents for the separation of Cr(III) from Cr(VI) and the preconcentration of Cr(III) from synthetic solutions and real samples: the first was the maleic acid-derivatised XAD resin (XAD-maleate) synthesised by a Friedel-Crafts reaction, and the second was a XAD-shellac sorbent synthesised by the impregnation of the natural polymer shellac on the Amberlite XAD-16 copolymer backbone. For both sorbents, Cr(VI) was adsorbed at the optimal pH of retention of Cr(III), *i.e.*, pH 4.0-5.5. The breakthrough capacity and preconcentration factors were 0.3 mg g^{-1} and 75, respectively, for XAD-shellac resin. All Cr determinations were made using the diphenyl carbazide (DPC) spectrophotometric method after oxidising Cr to Cr(VI), where necessary, and simultaneously with Flame Atomic Absorption Spectrometry (FAAS) for confirmation.

The XAD-shellac sorbent contained a number of hydroxy- carboxylic acids on its surface, namely jalaric acid and shelloic acid, enabling metal ion binding *via* the –COOH and –OH surface sites. The dynamic breakthrough and batch capacities of this sorbent for Cr(III) were 0.3 and 0.9 mg g^{-1}, respectively, indicating that the ion-exchange mechanism was prevalent in the dynamic mode, whereas in prolonged batch contact the surface sites were also capable of exerting their chelating effects. When the XAD-shellac resin was thoroughly washed with ammonium acetate solution prior to use in Cr speciation, the cationic (RH^+) surface sites were probably neutralised to yield free acetic acid ($RH^+ + CH_3COO^- \rightarrow R + CH_3COOH$) and the resulting resin did not retain CrO_4^{2-}. Thus, complete separation and speciation of Cr(III) from CrO_4^{2-} was possible using this sorbent.

The shellac-coated sorbent decomposed in alkaline solution (*i.e.*, above pH 7.5) and therefore the retained Cr(III) was eluted with dilute (0.025- 0.050 *M*) HCl. Thus, Cr(III) in admixture with Cr(VI) could be separated and recovered, without interference from the hexavalent state.

The XAD-maleate resin was capable of preconcentrating Cr(III) from brackish and artificially salted solutions as well as from seawater. The XAD-shellac resin, in turn, gave no satisfactory results for seawater, but could be efficiently used for synthetic and real electroplating wastewater. Excellent results were obtained in the case of Certified Reference Materials (CRMs), such as SO-2 soil, San Joaquin soil and BCR 145R sewage sludge, with a recovery ratio for Cr(III)/ Cr(VI) better than 98%.

LIQUID CHROMATOGRAPHY-UV SPECTROPHOTOMETRY METHOD FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY PURITY DETERMINATION OF AMIDINO DERIVATIVES

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Amidino derivatives and the corresponding amines have been analysed by means of the High Performance Liquid Chromatography (HPLC) method described hereafter. The amidino derivatives have been demonstrated to be effective agents in reducing NO and PGE_2 production by iNOS and COX-2. Such products might be promising drugs for the treatment of septic shock, rheumatoid arthritis, osteoarthritis, ulcerative colitis, insulin-dependent diabetes mellitus and asthma.

Several amino and amidino derivatives with the benzofuran framework have been synthesised in the Authors' laboratories and a simple and selective HPLC-UV spectrophotometry method has been developed for their HPLC purity determination. Chromatographic separation of the analytes was achieved on the reverse phase column Supelcosil LC-DP (150*4.6) mm 5µm, code 59150-U, constituted by the diphenyl ligand supported on microporous silica particles. The chromatographic mobile phase used was the 10 m*M* potassium dihydrogen phosphate buffer at pH = 2.1/methanol/acetonitrile, 25/27/48 (*v*/*v*/*v*) mixture and its flow rate was 0.45 ml/min.

The influence of substitutions in the 2- and/or 5-positions of benzofuran has been studied as regards the HPLC retention times of the analysed compounds and to their UV wavelengths of absorption in the 200-400 nm spectral range.

INHIBITION TYROSINASE ORGANIC PHASE BIOSENSOR FOR PESTICIDE ANALYSIS

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The tyrosinase biosensor, in the inhibition Organic Phase Enzyme Electrode (OPEE) configuration, allows triazine pesticides to be determined operating in an organic solvent (chloroform), in which the triazine pesticides tested are much more soluble than in water. This reduces the risk of underestimating the concentration of these pesticides due to the difficulty of dissolving them completely in the aqueous phase. The OPEE was made of PTFE and the enzyme was immobilized in kappa-carrageenan gel, *i.e.*, a type of immobilization that, as shown in previous research, gives particularly good results when operating in organic solvents. The method was first of all optimised by comparing two different possible experimental procedures. Both procedures are described in the literature and have been used in previous work with enzyme inhibition biosensors responsive to different kind of pesticides. In the former one, subsequent addition to the organic phase, at first of substrate (phenol), then of inhibitor (pesticide), were carried out. The latter procedure involved the recording of two successive biosensor responses to phenol, one in the absence and the other in the presence of the inhibitor after a so-called incubation period of the biosensor with it. In order to optimise the former analytical procedure, a set of measurements were initially performed to determine the optimal phenol concentration to be used for all subsequent measurements.

Another set of measurements was then carried out to determine whether the best solvent to use was water-saturated chloroform or the chloroform - *n*-hexane mixture 1+1 (*v*+*v*), also saturated with water. The investigation was limited to these two solvent mixtures as previous research carried out in the Authors' laboratory on this type of OPEE showed that in practice the best results in terms of method sensitivity and biosensor working life were generally obtained by using one or the other of these two solvent mixtures. The overall comparison of the experimental data obtained leads to the conclusion that the results obtained in triazinic pesticides analysis are in practice very similar when operating in the two different solvent mixtures considered. However, when operating in water-saturated chloroform a larger linearity range, a slightly better coefficient of correlation and a smaller confidence interval are obtained when the chloroform *n*-hexane mixture is used. All subsequent measurements were therefore carried out in water-saturated chloroform. Before carrying out the measurements using the latter operating procedure, it was however necessary to optimise the incubation time, which is defined as the contact time between inhibitor and enzyme prior to measurement. The comparison between the data obtained using the two operating procedures reveals that, by the latter one, a linearity range is obtained that is about two decades larger and has a better coefficient of correlation. It was consequently preferred to use this latter procedure to perform measurements on all examined triazine pesticides (Atrazine, Atrazine-desethil and Atraton). The biosensor method using a tyrosinase OPEE to determine triazine pesticides was also tested on real samples. To this end, different types of vegetal materials (field grass, olive and lemon samples) treated with triazine pesticides were chosen. The pesticide recovery, obtained using the biosensor method, was checked experimentally by analysing samples specifically treated previously with known quantities of atrazine. The results obtained indicates that the latter operating procedure again ensures better results than the former one, with a percentage of unrecovered pesticide of only 7-10%. At present, other triazinic pesticides are being tested, as well as other types of pesticides, such as dimethoate.

DEVELOPMENT AND VALIDATION OF AN ULTRASENSITIVE CHEMILUMINESCENT ENZYME IMMUNOASSAY FOR AFLATOXIN M1 IN MILK

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Aflatoxin M1 (AFM1) is a hydroxylated metabolite of Aflatoxin B1 (AFB1), formed when ruminants ingest feed contaminated with AFB1. AFM1 is then excreted in milk and, being relatively stable during milk pasteurisation or other thermal treatments, it can be also present in milk-derived dairy products, such as cheese and yogurt. Due to its hepatotoxic and carcinogenic activity AFM1 contamination represents a risk for human health, especially for children who are the major milk consumers. AFM1 legal limits, ranging from 0 to 1 μg/kg, have been established in various countries worldwide and, in particular, the European Commission limit for AFM1 in milk is 50 ng/kg.

Several methods for AFM1 determination have been developed and High Performance Liquid Chromatography (HPLC) with a fluorescent detector and enzyme-linked immunosorbent assays are mainly used in routine analysis. Previously developed enzyme immunoassays for AFM1 analysis using conventional colorimetric detection with chromogenic substrates allowed detection limits not lower than 5-10 ng/kg in milk to be reached. In addition, they usually required a 60- to 120-minute incubation time, as well as several analytical steps, which limited their extensive use for a rapid AFM1 screening.

A fast and ultrasensitive chemiluminescent enzyme immunoassay for aflatoxin M1 (AFM1) in milk samples has been developed and validated. The method is an indirect competitive type format involving the immobilization of an AFM1-bovine serum albumin conjugate on 384-well black polystyrene microtiter plates. AFM1 standard solutions were prepared in milk-based buffer and milk samples were analysed without any cleanup procedure. The Limit of Quantification (LoQ) was 1 ng/kg and the recovery ranged from 96 to 122%. The method is specific for aflatoxin M1 and other aflatoxins do not significantly cross react with the antibody.

Twenty-four milk samples were analysed and a good correlation was observed ($y = 0.98x + 1.71$, $r^2 = 0.98$) when the data were compared with a reference HPLC method with a fluorescent detector. The combination of a small incubation volume with rapidity and sensitivity of the chemiluminescent detection of the probe allowed a sensitive and low cost immunoassay to be developed, with which more than one hundred samples may be analysed in the same analytical session, thus representing a particularly attractive method for high-throughput screening of AFM1 in milk. In addition, the procedure can be automated using conventional instrumentation, easily available in laboratories where a continuous screening for AFM1 in milk is performed. In the near future, the method will also be validated for the determination of AFM1 milk-derived dairy products, such as cheese and yogurt.

A RECOMBINANT CELL-BASED BIOLUMINESCENT ASSAY FOR ANDROGEN BIOACTIVITY DETERMINATION IN ENVIRONMENTAL SAMPLES

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The recent increase in hormone-induced cancers and disorders of the reproductive tract in wildlife and humans has led to an urgent search for accurate and rapid methods for monitoring Endocrine-Disrupting Chemicals (EDCs) and evaluate their biological activity. These substances represent a heterogeneous class of molecules either steroidal or not, sharing the ability of interfering with the endocrine system *via* nuclear receptor signaling pathways. Up to now, little attention has been dedicated to the development of assays for androgen-like compounds.

A yeast-based bioassay to measure androgen bioactivity in environmental samples has been developed and validated for the detection of androgen-like compounds according to their activity. The biosensor is based on recombinant *Saccharomyces cerevisiae* cells expressing the human androgen receptor (hAR) and containing the sequence Androgen Response Element (ARE) which drives the expression of *Photinus pyralis* luciferase. The use of a luciferase without the peroxisomal targeting sequence abolished luciferase import into peroxisomes, resulting in high levels of light emission without interfering with normal host physiology. In the presence of androgenic compounds, hAR, which is a ligand-activated transcription factor, moves into the nucleus and binds ARE sequences, resulting in luciferase expression, which is directly proportional to the androgenic activity of the sample.

The androgen bioassay is performed in a 96-well microtiter plate format and a recombinant yeast strain constitutively expressing luciferase is used as control for signal correction according to cell vitality and matrix aspecific effects. The recombinant assay requires only 2 h 30 min of incubation time of the cell suspension with 10 μl of sample or the analyte in solution. Luminescence measurements are then performed by simple addition of D-luciferin without cell lysis or washing steps, thus improving the assay analytical performance.

The yeast biosensor showed a limit of detection of 0.05 n*M* for testosterone, comparable or even lower to that of previous androgen reporter gene assays based on human cell lines with a dynamic range from 0.05 to 1000 n*M*. The biosensor responds to other androgen-like compounds such as *trans*-androsterone and androstenedione; in addition, the specificity of the bioassay was further demonstrated by the ability of antiandrogens to suppress luciferase induction.

Effluents before and after treatment in municipal sewage treatment plants were analysed with this bioassay and androgenic activities decreased markedly as wastewater progressed through the plants with removal efficiencies ranging from 30 to 90%. These results were consistent with data previously obtained through chemical analysis of the same samples.

A RAPID CHEMILUMINESCENT IMMUNOASSAY FORMAT FOR SIMULTANEOUS DETECTION OF *E. COLI O157:H7*, *YERSINIA ENTEROCOLITICA*, *SALMONELLA TYPHIMURIUM* AND *LISTERIA MONOCYTOGENES* PATHOGEN BACTERIA

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The identification of bacteria in classical microbiology procedures typically requires two to three days to obtain results. Thus, rapid and reliable analytical methods are required in order to control food safety along the entire food chain (food production, processing, storage and distribution) and to prevent food-borne diseases.

Polyclonal and monoclonal antibodies were produced by immunising rabbit or balb/c mices, respectively, with heat inactivated and sonicated ATCC reference strains and used to develop a simple and rapid immunoassay for simultaneous detection of four of the major food-borne pathogens (*Escherichia coli O157:H7*, *Yersinia enterocolitica*, *Salmonella typhimurium* and *Listeria monocytogenes*) in food. The peculiarity of the monoclonal antibodies produced was their ability to recognise all the strains of each bacteria species without any cross-reactivity with other species.

To achieve the simultaneous detection of the four pathogens in the same sample, a new black polystyrene 96x4-well microtiter plate format was developed. The microtiter plate, which has a conventional external size (12x7.5 cm), consists of 96 main wells (100 μl), each of them divided into four internal subwells (15 μl). Using this innovative format, a multiplexed sandwich chemiluminescent enzyme immunoassay (CLEIA) method was developed, which involves the immobilization of four monoclonal antibodies, each specific for one bacterium, in different subwells of the main well. Antibody immobilization was obtained by physical absorption and coated plates were stable for up to six months if sealed and stored at 4 °C. When aqueous samples were added to the main well and incubated (1 h, 37 °C), bacterial cells were able to specifically bind to the corresponding monoclonal antibody and thus be captured in one of the four subwells. Subsequently, a mixture of peroxidase-labeled polyclonal antibodies was added and, after 1 h at 37 °C incubation, the peroxidase activity was measured by means of an enhanced luminol-based chemiluminescent substrate and a low light CCD imaging detector. Use of an ultrasensitive CCD camera allowed the spatial distribution of the light signal within the subwell area to be imaged and quantified. Thanks to spatial resolution of the imaging system and to the high detectability of the peroxidase chemiluminescence detection, sensitive and rapid quantification of each bacterial species was performed, in low reaction volumes (15 µl /subwell), without any cross-talk of the chemiluminescent signal among adjacent subwells.

The detection limits of the developed method were found to be in the range 104-105 cells/ml for all bacterial species and calibration curves were linear in the range between 104 to 107 cells/ml. The within-run and between-runs coefficients of variation were below 15%. Accuracy of the method was determined by comparison with conventional culturing methodology on selective media and recovery resulted to be between 90 and 120%.

In conclusion, the multiplexed sandwich CLEJA allows for the simultaneous quantification of four pathogen bacteria with reduced assay time, sample and reagents consumption with respect to conventional single analyte immunoassays and classical microbiology procedures.

ANALYTICAL APPROACH BASED ON MASS SPECTROMETRY TECHNIQUES FOR THE STUDY OF IMPURITIES IN BIOTECHNOLOGICAL DRUGS: RASBURICASE

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The safety of a drug product depends not only on the toxicological properties of the active drug substance itself, but also on the presence of impurities and their metabolism. Analytical monitoring of impurities in new drugs is a key component of the recent guideline issued by the International Conference on Harmonization (ICH). The determination of absolute, as well as relative, purity presents considerable analytical challenges and the results are highly method-dependent. Consequently, the purity of the drug substance and drug product is assessed by a combination of analytical procedures. The analytical problem becomes even more complicated when the drug is a functional protein such as an enzyme. In this case the drug characterisation should include not only the assessment of the drug chemical purity, but also of its enzymatic activity and, more generally, its functional activity. This implies a careful choice of sample preparation steps or separation methods because denaturation or other processes affecting the enzyme activity (such as aggregate or metal adduct formation, loss of structural metals or prosthetic groups) must be avoided.

In the present work a comprehensive analytical approach for the identification and characterisation of impurities in protein drugs is reported. This analytical approach was applied to the case of uricase (urate oxidase), an antihyperuricemic drug, to design and evaluate a comprehensive, systematic and general approach to the analysis and characterisation of impurities in functional proteins obtained by processes based on DNA recombinant technology. A recombinant urate oxidase expressed in *Saccharomyces cerevisiae* and commercialized as Rasburicase by Sanofi-Synthelabo has been analysed in comparison with a reference urate oxidase obtained from Sigma-Aldrich (recombinant urate oxidase from *Candida sp*. expressed in *E. coli*).

Samples were first analysed by Matrix-Assisted Laser Desorption Ionization - Time-Of-Flight Mass Spectrometry (MALDI-TOF-MS), with or without pre-separation *via* SDS-PAGE or 2D-PAGE, which are conventional separation techniques for the assessment of protein purity. Further characterisation was then performed by combining conventional Reverse Phase High Performance Liquid Chromatography (RP HPLC) with UV/Vis and ElectroSpray Ionization Quadrupole Time-Of-Flight Mass Spectrometry (ESI-Q-TOF-MS). To finally characterise not only the chemical identity, but also the enzymatic activity of the sample components, an innovative approach was developed which is based on flow field-flow fractionation, a non-denaturing separation technique, with chemiluminescence detection.

SYNTHESIS AND CHARACTERIZATION OF Cu(II) COMPLEXES OF VALACYCLOVIR

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Valacyclovir is the L-valyl ester prodruct of acyclovir. Valacyclovir is converted rapidly and virtually completely to acyclovir after oral administration in healthy adults. This conversion is thought to result from first-pass intestinal and hepatic metabolism through enzymatic hydrolysis. The drug has been approved for treating herpes viruses. To date, there have been no reports in the literature concerning the complexation of valacyclovir with Cu(II).

In this study, the complexation between Cu(II) and the antiviral drug valacyclovir was studied in aqueous methanol media at molar ratio from 1:1 and 1:10. The complex was characterised on the basis of analytical data, magnetic moments, Atomic Absorption, Spectrometry IR and electronic spectral data. The molar conductivitiy of the complex at room temperature was measured. The thermal properties of the complex was studied by Differential Thermal Analysis - Thermogravimetry (DTA-TG) techniques.

Oral session 3

The manufacturers’ views

Chairperson
I.B. Brenner

APPLICATIONS OF A NEW CCD SIMULTANEOUS INDUCTIVELY COUPLED PLASMA SPECTROMETER

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Various applications have been performed using a new simultaneous CCD spectrometer. The performance on this new spectrometer is described and supported with various figures of merit on a variety of elements and matrices. The spectrometer features good optical resolution and this can be advantageous for a range of higher elemental wavelengths, including the rare earth elements that pose serious analytical problems due to the similarity of their complex spectra.

Application work to be described includes: bovine liver, hair and effluents, all giving good agreement with Certified Reference Materials (CRMs) values as well as results on other complex matrices including different alloy analysis and organics using this new Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) instrument. The design criteria for the research and development programme of this new CCD ICP-AES instrument are finally illustrated.

DINAMIC REACTION CELL INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY IN THE CLINICAL AND BIOLOGICAL FIELD OF APPLICATION

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Clinical and biological analysis is important for assessing exposure levels in different fields such as occupational (Cr, Ni, Pb, V), environmental (Cd, Hg, Pb) and health (Al) applications. Other elements need also to be determined precisely and accurately because some of them (Se) are essential, although may have toxic effects at too high levels, while the importance of not well known elements (Mo) needs to be evaluated.

Standard Quadrupole Inductively Coupled Plasma Mass Spectrometry (Q-ICP-MS) provides very good results for several elements in clinical and biological matrices, while other important elements are difficult to determine because affected by interferences related to plasma background or matrix, *i.e.*, ^{52}Cr by ^{40}Ar^{12}C, ^{75}As by ^{40}Ar^{35}C1 and ^{51}V by ^{35}Cl^{16}O, thus resulting in a reduction in the ICP-MS power of detection.

The Dynamic Reaction Cell (DRC) technology overcomes these interferences *via* a process called “chemical resolution”, where a reaction gas is used to selectively remove interfering species before they enter the quadrupole analyser. This approach greatly simplifies and improves the overall analytical procedure, permitting the determination of interfered elements with better accuracy and detection limit.

ICP-MS is also easily coupled to chromatographic techniques such as Ionic Chromatography (IC) or High Performance Liquid Chromatography (HPLC) for element speciation, to allow for the evaluation of the different chemical forms of the elements (due to their different toxicity levels) or to study how elements are bonded and distributed in body fluids.

HELIUM COLLISION MODE. THE ULTIMATE SOLUTION FOR COMPLEX, UNKNOWN SAMPLE MATRICES

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The rapid acceptance of collision and reaction cell technology in conventional Quadrupole Inductively Coupled Plasma Mass Spectrometry (QICP-MS) instrumentation is due to the improvement in the measurement of interfered elements, which previously proved difficult to analyse at the required levels in complex sample matrices.The elements affected can be divided into two groups: those that suffer from plasma-based interferences and those that suffer from matrix-based interferences. This is because plasma-derived interferences are relatively constant and easy to predict, with one, high intensity interferent typically being the main target. Matrix-derived interferences, on the other hand, may be complex (several interfering species occurring at the same analyte mass), unpredictable and can vary by several orders of magnitude between ostensibly similar samples.While research laboratories may have the opportunity to evaluate each sample type individually to identify the most critical matrix-based interferences, this approach is not practical in the routine laboratory. Routine laboratories require a matrix-independent interference removal technique that can be applied to as many interfered elements as possible, in order to make maximum advantage of the collision/reaction cell.

Operating the cell in the collision - only mode, using pure He as the collision gas - has been found to be a reliable and widely applicable way to remove matrix-based interferences. The use of an inert gas - pure He - means that no new interferences are formed as a result of reaction with sample matrix or analyte and no matrix-dependant loss of analyte or internal standard (by reaction) can occur. The interference removal efficiency of the He collision mode is increased by the use of Kinetic Energy Discrimination (KED), which itself is dependant upon precise ion energy control in the interface. This is achieved by the use of a physical shield to isolate the plasma from the RF field generated by the ICP coil. The interference removal capability of these consistent cell conditions is illustrated using spectral scans (as applied to the screening of unknown samples), quantitative recovery data from synthetic, high matrix samples and a simulated, routine environmental sample batch. In addition, isotope selection, internal standard use and the elimination of interference correction equations is discussed.

A FAST ANALYTICAL METHOD FOR Hg DETERMINATION IN SOILS AND SEDIMENTS, WITHOUT SAMPLE PREPARATION

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While the total amount of Hg on the planet has remained constant, the amount released in the biosphere has dramatically increased over the past century. Emissions from fossil fuel combustion are responsible for generating the greatest amounts of Hg in the atmosphere. The metal then enters the food chain through soil contamination and bioaccumulation in fish and animal tissue. Other contamination sources include medical and hazardous waste incineration, industrial waste and even broken thermometers and manometers.

The risk posed by contamination makes accurate detection extremely important. While traditional Cold Vapour (CV) methods work well, they are difficult, highly time-consuming and generate their own kinds of hazardous waste. Thus, another method needed to be developed that would generate accurate and repeatable results quickly, simply and waste-free. Milestone's DMA 80 Direct Hg Analyser effortlessly analyses liquid and solid samples with no sample preparation and no waste disposal. It automatically processes 40 samples in about 4 hours from start to finish.

A user-friendly device weights, controls the analysis and processes data with built-in report generation and networking capabilities. Solid and liquid samples are weighed and introduced into the sample boat. The sample is initially dried and then thermally decomposed in a continuous flow of oxygen. Combustion products are carried off and further decomposed in a hot catalyst bed. Mercury vapours are trapped on a gold amalgamator and subsequently desorbed for quantification. The Hg content is determined using Atomic Absorption Spectrometry (AAS) at 254 nm. Several applications for different types of Certified Reference Materials (CRMs) are described and compared.

OPTIMISATION OF PARAMETERS FOR THE ANALYSIS OF THE DECA-BROMINATED DIPHENYLETHER (BDE-209) BY GAS CHROMATOGRAPHY HIGH RESOLUTION MASS SPECTROMETRY

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Brominated diphenyl ethers (BDEs) are widely used as flame-retardants in electronics industry. However, for a number of reasons the mass spectrometric analysis of BDEs by high resolution magnetic sector mass spectrometry is more complicated than, *e.g.*, analysis of polychlorinated dioxins and furans, namely: *i*) a high mass range from m/z 248 (mono-BDE) to m/z 960 (deca-BDE) is required, therefore demanding a reference compound that provides sufficient intensity on high m/z ions; *ii*) large electric jumps are necessary for window defining measurements, which demands for a stable and rugged electric mass calibration; and *iii*) the Deca-BDE is thermally labile and thus requires optimised chromatographic methods. This study shows how much different chromatographic parameters can influence the analytical sensitivity for the thermolabile deca-BDE. Furthermore, a method setup is presented, which combines optimum sensitivity for the deca-BDE and at the same time good separation efficiency for mono- to hepta-BDE.

A number of chromatographic parameters and their effects on the analytical response for the deca-BDE have been investigated in this study. It could be shown that Gas Cromatography (GC) oven temperature at the elution time of the compound is the decisive factor for the sensitivity of the deca congener. The lower the temperature for elution, the better the response. Column flow has also an important effect on sensitivity. By increasing the column flow the elution temperature on a short column could be further decreased. Data are presented to support these assumptions.

As a rule of thumb, it can be stated that the lower the elution temperature for the deca-BDE, the better the sensitivity. This is also the main factor for a shorter column giving better analysis results. Comparing split/splitless and PTV injectors for BDE analysis it can be concluded that the PTV injector using an appropriate temperature programme provides better sensitivity and stability for the deca-BDE, whereas for the remaining BDE congeners the response is comparable on both injectors.

As a conclusion from the results presented above, a dual column setup was employed. Two GC columns were introduced into the mass spectrometer by means of a custom-designed Y-piece adaptor. In this way the advantages of both a short and a long column can be combined on one analytical system resulting in good sensitivity for the deca-BDE and good separation efficiency for all remaining BDE congeners. It has been shown that the optimisation of chromatographic parameters is crucial for good sensitivity when analysing deca-BDE. The elution temperature of the deca congener from the column proved to be the decisive factor for a successful analysis at lower concentrations.

Oral session 4

Quality aspects

Chairperson
M. Veber

MEETING THE NEED FOR INTERCONTINENTALLY UNDERSTOOD CONCEPTS AND TERMS IN CHEMICAL MEASUREMENT: THE 2005 EDITION OF THE INTERNATIONAL VOCABULARY OF BASIC AND GENERAL TERMS IN METROLOGY (VIM)

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Unambiguous and consistent concepts and terms such as measurand, metrological traceability, measurement uncertainty, comparability of measurement results, target measurement uncertainty *etc.* must govern the description of results of chemical measurements in order to enable a valid comparison of measurement results. That is not yet the case as numerous workshops over the last decade have shown world-wide and as chemical literature continuously displays. For international trade in food and feed to be fair, for border-crossing implementation of environmental regulations to be the same for all parties concerned, for interchangeability of results of clinical measurements to become a reality, for any border-crossing interpretation of measurement results in chemistry to become possible, well understood and mutually accepted, concepts and terms are essential. Similarly, their translations from one language – English – to 30-40 other languages must be carried out and fixed unequivocally. Countries using English as common language have not yet fully realized that they are at a considerable advantage over countries where such translated terms describing concepts may not yet be available, let alone understood and accepted. These countries are therefore at a considerable disadvantage in all cases where chemical measurements are involved.

A number of ambiguities in the definitions and terms are described which illustrate the importance of the ongoing revision (1997-2005) of the International Vocabulary of Basic and General Terms in Metrology (VIM), especially since chemical measurements will enter the VIM for the first time in history with terms such as: *measurand, measurement unit, measurement scale, measurement result, metrological comparability, metrological traceability* (including "*to the SI"*), *measurement uncertainty, target measurement uncertainty*. It is concluded that the ongoing revision of the VIM is of primordial importance for good understanding within and between the measurement communities world-wide.

To prevent War, define your Words [Kongfutze 551-479 B.C.]

NEW CERTIFIED REFERENCE MATERIALS FOR IMPROVING THE QUALITY OF SPECIATION ANALYSIS

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The recognition that quantification of the different forms of elements in samples of relevance to the environment, food and health, is more important than the determination of the total elemental level has led to an explosive growth in what is now known as speciation analysis. This lecture will review some of the reasons for the growth in such analysis and describe some of the methods currently in use for speciation analysis.

One of the consequences of the development of new analytical methodologies and their impact upon regulatory régimes is the need to assure the quality of analysis. While there are now a number of different approaches to trace element speciation, which will be illustrated in the lecture, the best way of validating an analytical method is by the use of Certified Reference Materials (CRMs). The European Union has played a leading role in the development of CRMs and these are now available, *e.g.*, for Pb and Sn species. There is clearly a need to extend the range of certified reference materials available to include some of the new challenges presented to analytical science. In a recent EU funded feasibility study into the preparation and certification of materials to validate speciation measurements, five new materials were developed. Chicken meat, rice and marine fish for As species and flour and selenized yeast for Se. The importance of these samples is discussed in both environmental and health terms as is the identification and collection of these samples. A detailed description is given of the preparation, stability and homogeneity tests of the candidate materials and the analytical methods used by the eighteen participating laboratories are outlined. The majority of expert laboratories used coupled chromatography with atomic or mass spectrometry; particularly the coupling of High Performance Liquid Chromatography (HPLC) with Inductively Coupled Plasma Mass Spectrometry (ICP-MS). A range of analytical approaches gave comparable results.

It was possible to agree values of: arsenobetaine in fish (plaice) of 92 ± 5 $\mu g\ g^{-1}$ (total As 43 ± 5 $\mu g\ g^{-1}$); of dimethylarsonic acid (DMA) in rice of 87±24 ng g^{1} (total As 196±18 ng g^{-1}); of total As in chicken of 170±10 ng g^{-1} with an indicative DMA content of 150 ng g^{-1}; total As in a soil of 2250±140 $\mu g\ g^{-1}$ which contained As (lll) at 142±20 $\mu g\ g^{-1}$ and As(V) at 1600±600 $\mu g\ g^{-1}$; of total As in a second soil of 610±29 $\mu g\ g^{-1}$ which contained As(lll) at 18±5 $\mu g\ g^{-1}$, As(V) at 515±27 $\mu g\ g^{-1}$ and DMA at 54±8 $\mu g\ g^{-1}$; of total Se in flour of 600±50 ng g^{-1} with an indicative value for selenomethionine of 700 ng g^{-1} and for the selenised yeast of 1380±100 $\mu g\ g^{-1}$ for total Se with selenomethionine at 2200±260 $\mu g\ g^{-1}$.

Future prospects for speciation analysis in Europe are discussed and the latest developments for the European Virtual Institute for Speciation Analysis (EVISA) are described.

INTERLABORATORY STUDY FOR OCHRATOXIN A (OTA) DETERMINATION IN COCOA POWDER SAMPLES

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An interlaboratory study aimed at validating a High Performance Liquid Cromotography (HPLC) method of analysis for the determination of ochratoxin A (OTA) in cocoa powder was performed. The method was tested at three levels of OTA covering the range in which presumably European regulatory limits could fall. Ochratoxin A was extracted from samples by blending with aqueous solution of bicarbonate, diluting with a solution of phosphate buffer saline, filtering and cleaning up by immunoaffinity column containing antibodies specific to OTA. After washing the immunoaffinity column, OTA was eluted with methanol, identified by reversed-phase HPLC and quantified by fluorescence detection. Five coded materials (blank and naturally contaminated samples) were shipped to 31 laboratories (25 national and six European) together with OTA calibrant and spiking solutions. From the results submitted, the relative standard deviation for repeatability (15-31%) and reproducibility (29-40%) showed reliable results adequately matching the criteria suggested by the European Committee for Standardization (CEN) for the analysis of mycotoxins, as shown by HORRAT values for all three levels of contamination. This validated method makes it possible to detect OTA in cocoa powder at μg/kg levels, representing, therefore, a useful tool for the control of this kind of foodstuff, in accordance with the upcoming community legislation.

IMPROVING THE QUALITY OF ANALYTICAL MEASUREMENTS IN SUPPORT OF THE IMPLEMENTATION OF THE WATER FRAMEWORK DIRECTIVE IN THE EUROPEAN LABORATORIES

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The project Screening Methods for Water Data Information in support of the implementation of the Water Framework Directive (SWIFT-WFD), funded by the European Commission (Contract no. SSPI – CT – 2003 – 502492) for three years started on the 1st of January, 2004. The main objective of the project is to support the successful implementation of the WFD which strongly depends on the quality of monitoring data and their comparability from river basin to river basin. This objective requires primarily, but not only, the development, validation and dissemination of rapid, affordable and user friendly measurement techniques. One of the activities of the project is the production of three sets of Reference Materials (RM) and the corresponding calibrants for the quality control of the analysis of the priority pollutants listed in the Directive, as well as the organisation of third Proficiency Testing Schemes (PTSs) among European laboratories experienced in the analysis of these analytes.

The first set of materials consist of four river water reference materials (two at natural and two at fortified analyte levels) for several trace elements and major components. The batch sample was taken from the river Meuse, the Netherlands, and transported to the Institute for Reference Materials and Measurements (IRMM) for the preparation of Reference Materials (RMs.) The spiking solutions, to prepare the RMs at fortified levels, and the calibrants were prepared by the University of Barcelona.

The first PTS focused on the assessment of laboratory performance in the field of major component and trace metals determination in surface water – at natural and fortified concentration levels – applying classical measurement techniques. The participating laboratories were provided (free of charge) with: *i*) two quality control (reference) materials at natural concentration level - one for major component analysis (target measurands: Na, Ca, Mg, total P, chloride, sulphate, nitrate, pH and conductivity); and one for trace elements (target analytes, As, Cd, Cu, Ni, Pb and Zn – mandatory; Al, Cr, Mn and Se – optional); *ii*) spiking solutions to produce the two quality control (reference) materials at fortified concentration levels; *iii*) a set of suitable calibrants; and *iv*) the necessary technical assistance (guidelines and electronic formats for data reporting).

The RMs, calibrants and spiking solutions were shipped to the participating laboratories between mid and end of September 2004. The deadline for results submission was the 3rd of November, 2004. All together 58 laboratories from 21 European Countries received samples to participate in the first SWIFT-WFD PTs and 56 laboratories submitted results. 48 Laboratories analysed the test material for trace elements and 47 for major components. The technical evaluation meeting of the PTS took place on the 2nd and 3rd of December, 2004 in Rome. The results of the first SWIFT-WFD PT, along with the introduction of the main conclusions of the Rome meeting, are presented and briefly discussed.

VIRTUAL INSTITUTE OF REFERENCE MATERIAL: THE “MEETING PLACE” FOR ALL REFERENCE MATERIAL USERS AND PRODUCERS

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The aim of the European Virtual Institute for Reference Materials (VIRM) is to offer a meeting place for the European Reference Materials (RMs) community. It actively involves a consortium of experts from all over Europe, covering R&D organisations, governmental and private laboratories, RMs producers and industry. The goal is to facilitate dissemination of information and advice, know-how and help on RMs and related fields, thus evolving toward an indispensable tool in Quality Control for analytical measurements. The innovative character of VIRM consists in its virtual structure, combining a minimal logistic infrastructure with the maximal effectiveness of a European-wide ICT network. The scope of VIRM covers all European countries. The development of VIRM is funded by the European Community within the framework of the 5th Research Framework (Contract: G7RT-CT-2002-05104); the project, started on 1st January 2003, comprises a consortium of 22 Core Group (CG) Members, who are specialists from research, governmental and private organisations with outstanding complementary experience and expertise in the field of RMs. On November 2004 the VIRM no-profit association has been established as a legally independent organisation under the law of Luxembourg. The Executive Committee of the Institute has been appointed as formed by Roberto Morabito (ENEA - IT) as Chairman, Iain Weir (OPTIMAT, UK) as General Secretary and Kees Kramer (MERMAYDE, NL).

VIRM extends to everybody who has to maintain high level of quality of work. Correct measurements are essential to maintain and control quality of products and processes in research, manufacturing and trade. They re-assure to fulfil national regulations and thus support industry in developing a sustainable economic growth. VIRM offers through its website, a broad range of useful tools in the field of Reference Materials and Quality Control. Members will have unlimited access to the common use features, with regular newsletters and information on both users and producers of RM’s. These will include: searchable database of RMs, library of position papers; a discussion forum on RM hot topics; network links to related projects; glossary, guidelines and events related to Reference Materials, QA/QC, routine analysis, information on producers of RM’s.

The VIRM services include: i) the website, a key link between the VIRM members. It offers extensive search facilities (persons, projects, RMs etc) and is the central place for disseminating information (www.VIRM.net); ii) the newsletter periodic update via e-mail with the latest information, news, conferences, new RMs from the community; iii) The helpdesk (based in Italy). This is the only physical entity of the Virtual Institute. The staff takes care of continuous updating of the website, but they are also available for Your questions. They forward your queries to the specialist member of the VIRM (info@VIRM.net); iv) the National Contact Points (NCPs). They are established in all European countries, disseminate information, are ready for your questions and speak the local language.

Membership allows for access to all VIRM services, newsletter subscription, use of databases and download facilities. Further information and appliation forms can be obtained from the website www.VIRM.net.

Oral session 5

Food analysis

Chairperson
Gy. Záray

ELEMENT FINGERPRINTING OF MARINE ORGANISMS: A CHALLENGE TO THE MULTIELEMENT CAPABILITIES OF QUADRUPOLE INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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In the assessment of seafood quality and safety, the determination of the multielement profile of fish and shellfish species is useful for different purposes. The element fingerprints of sedentary organisms can be used to identify pollution hot spots and trace point sources of metal(loid) contamination. Monitoring of multielement profiles allows for the identification of temporal trends, verification of the effectiveness of pollution control strategies and early warning of adverse impact in vulnerable areas, thus providing valuable information for the management of edible marine resources of inshore fisheries. On the other hand it is well known that, irrespective of the presence of pollution sources, some marine organisms can accumulate metals and other elements whose potential toxicity is of particular concern. Since several species used for human consumption are poorly characterised in terms of their trace element composition, multielement screening is valuable to identify seafood items that requires further investigation (*e.g.,* speciation studies) in order to assess possible health risks for regular consumers of such commodities. Element fingerprinting has a potential in the area of food authentication as well and can find useful application in the fishing sector, *e.g.,* for the identification of commercial frauds such as product substitutions with similar items of lower economic value.

Multielement analysis of seafood by Quadrupole Inductively Coupled Plasma Mass Spectrometry (Q-ICP-MS) is challenging due to significant matrix-induced spectral and non-spectral interferences. In this study, a systematic investigation of polyatomic interferences was carried out in the light of the chemical composition of marine organisms belonging to different taxa. Polyatomic interferences arising from the major element constituents of seafood matrixes (*i.e.*, C, Ca, Cl, K, Mg, Na, P, S) have been quantified and suitable solutions to overcome their effect, including changes in the sample introduction system and chemical resolution by a Dynamic Reaction Cell (DRC), have been identified. Acid effects and other matrix effects originating from fish and shellfish samples submitted to closed-vessel microwave digestion have also been investigated in order to identify the best analytical conditions for reliable multielement determinations. The accuracy of measurements was evaluated by the analysis of three Certified Reference Materials (CRMs), namely BCR CRM 278R (Mussel tissue), BCR CRM 422 (Cod muscle) and DORM-2 (Dogfish muscle).

Selected applications of the developed analytical approach are presented to show how multielement analysis can be employed to address quality and safety issues of aquiculture and fishery production: i) comparison of three mussel farming sites in the Venice Lagoon by means of multivariate analysis of mussel element fingerprints (As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, Sn, V, Zn); *ii*) characterisation of the multielement profile (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, Sn, V, Zn) of some species of predatory fishes caught in the Mediterranean Sea; *iii*) simultaneous determination of selected toxic or allergenic elements (As, Cd, Ni, Pb, Sb, Tl) in Mediterranean edible gastropods as a part of a study on seasonal variations in metal(loid) tissue concentrations of mollusc species.

ORGANOTINS SPECIATION IN SOIL AND CULTIVATED VEGETABLES: PERSISTENCE AND PLANT UPTAKE

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Organo-Tin Compounds (OTCs) are widely used for their biocide effects in various agricultural or industrial activities. Their intensive utilisation leads to their environmental presence particularly in sewage sludge which is valorised by spreading on agricultural soils. Among the OTCs species, the trisubstituted ones such as tributyltin (TBT) and triphenyltin (TPhT) are the most used and toxic. Hence, it is important to understand the fate of these compounds in farming soil and get data about their persistence, phytoavailability and plant uptake. This information is essential to evaluate the potential food risk and to propose fit-for-purpose agricultural management.

The present study focuses on the evaluation of the influence of various chemical parameters on the persistence of TBT and TPhT. These compounds were added in soil through contaminated sewage sludge to simulate an agricultural practice very used in France. Lettuces and French beans were cultivated in the same soil in order to assess the plant uptake. Considering the variable toxicity and physical-chemical properties of Sn species, a particular attention was given to speciation.

The investigation was carried out by a chemometrics design. Concentration of TBT and TPhT, quantity of sludge and pH are the factors taken into account for the experimental designs. Vegetable cultures were achieved in greenhouse and degradation study was done under biotic conditions in an incubator at 28 °C. The speciation was performed either in shoots or roots of vegetables. The results confirm a significant transfer from soil to leaves and fruits at some μg (Sn)/kg concentration levels.

Contaminant accumulation is dependant on the quantity of sludge added in soil. Degradation kinetics was evaluated and OTC half-lives were calculated. The TBT initial concentration in the soil appears to be the key factor on TBT degradation whatever the duration of incubation is. TBT persistence is important (half-lives over 50 days) probably due to his biocide effect, while important TPhT degradations occur (half-lives reached after 7 days). Biotic degradation of these two compounds seems to be ascribable to different micro-organisms. Generally, OTC fate in soil-plant system is linked to the type of compounds and physical-chemical mechanisms occurring in the soil and liquid/solid interface. Finally, these results highlight the dependence between culture conditions, OTC persistence in soil and contamination levels of the cultivated vegetables.

QUANTITATIVE Se SPECIATION IN WILD AND SELENISED MUSHROOMS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY INDUCTIVELY COUPLED PLASMA – OCTAPOLE REACTION CELL – MASS SPECTROMETRY USING POST-COLUMN ISOTOPE DILUTION ANALYSIS

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Selenium is an essential element for animals and humans, playing important biological roles as antioxidant, as regulator of thyroid hormone metabolism and as anti-carcinogenic agent. The main dietary sources of the element are fish, seafood and certain vegetables. Among vegetables, garlic, Brazil nuts and mushrooms contain very high Se levels. The chemical form in which Se is ingested dictates its bioavailability. Therefore, the differentiation of the chemical Se species present in the food products is necessary to identify the effective sources of nutritional Se.

As very limited information exists so far on the levels of different Se species in foodstuffs, accurate and precise analytical methodologies for the quantitative speciation analysis of Se in this kind of samples are mandatory. In this sense, Se speciation studies in biological materials are mainly carried out by High Performance Liquid Chromatography (HPLC) coupled with Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

However, the accurate quantification of the separated Se species is not always possible by the "traditional" techniques (such as external or standard addition calibration methods), as most of them are unknown (therefore, no commercially available standards for calibration can be used). As an alternative, the application of Isotope Dilution Analysis (IDA) using the species-unspecific spiking mode on-line with the HPLC separation allows for the accurate quantification of the heteroatom measured in the compound, even in unknown species.

The aim of this work was the comparison of Se species naturally occurring in wild (*Agaricus*, *Lepista luscina*, *Macrolepiota procera* and *Boletus luridus*) and selenised (*Agaricus bisporus*) mushrooms. The study involved the use of aqueous and/or enzymatic extraction for solubilisation of Se. The corresponding extracts were analysed by HPLC-ICP-ORC-MS using post-column IDA. Results showed marked differences not only in the total Se contents, but also in the Se species found in the different types of mushrooms investigated. Selenomethionine was detected in both wild and Se-enriched mushrooms with a number of unknown selenocompounds.

IS THE REDUCTION OF ACRYLAMIDE BY NEW FOOD PROCESSING TECHNIQUES BENEFICIAL IN TERMS OF LOSS OF OTHER COMPOUNDS?

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Acrylamide is an industrial chemical used for the manufacture of polyacrylamide or its copolymers. In April 2002, the Swedish National Food Authority reported the finding of elevated levels of acrylamide in heat-treated potato products and other baked goods. Despite the fact that acrylamide has been produced commercially for many years for technical use and that its toxicological properties have been well investigated, the discovery of acrylamide formation in cooked food was completely new. Following this discovery, a world-wide surveillance of this substance in various food products was immediately started. In addition, a joint effort was undertaken by the national food authorities and the food industry in order to gain a better understanding of the mechanisms of formation and of contamination levels of acrylamide in food. Some initiatives have already been taken by the industry in order to decrease the content of acrylamide in food.

The underlying mechanism leading to the formation of acrylamide is still not fully understood. It is assumed that it derives from the reaction between reducing sugars and proteins/amino acids (mainly asparagine), the so-called Maillard reaction. It is also well-known that some antioxidant compounds, namely melanoidins, are also products of the Maillard reaction. Theoretically, every measure taken to decrease the concentration of acrylamide in food would also induce a reduction in the formation of melanoidins and consequently in the antioxidant activity of the relevant food. The positive influence of antioxidants decreasing the risk of cancer and other non-transmissible diseases has been known for years.

This work shows the results of a systematic study in which butter cookies were prepared changing some parameters, such as baking temperature, cooking time and amount and type of sugar added. Both concentration of acrylamide and antioxidant activities of the final products were measured and compared.

DIOXINS AND DIOXIN-LIKE COMPOUNDS IN FOOD PRODUCTS

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Several studies have shown that the main cause of human exposure to environmental pollutants such as dioxins and dioxin-like compounds is the diet. During the last years, some events have pointed out the presence of dioxins and dioxin-like compounds in European food commodities. As a result, research focused on risk for humans. The presence of dioxins and dioxin-like compounds in food can cause adverse effects on organisms; effects on immune system, liver and endocrine system have been shown. In each matrix dioxins and PCBs are not detected as single compounds, but as complex mixtures; moreover, congeners are not all, or in the same way, toxic.

To allow exposure data to be compared, the analytical results are expressed as Toxic Equivalency Factors (TEFs) as referred to the strongest substance of this group of compounds, 2, 3, 7, 8-TCDD. In this investigation the concentrations of the aforesaid pollutants, in a wide range of food products of the standard Italian diet have been assessed. The gas-cromatographic analysis has shown the presence, in most samples, of the mono-*ortho* PCBs congeners and non-*ortho* PCBs, or coplanars. Only in some samples it has been possible to assess the concentrations of dioxins and furans, since most often they are below the detection limits. In the vast majority of the samples the concentrations of the examined compounds are in the order PCB mono-*ortho* > PCB non-*ortho* > PCDDs and PCDFs. The highest concentrations are those concerning the fish products; also the dairy products showed PCBs concentrations slightly higher than the other products.

LEVELS AND DISTRIBUTIONS OF PCDD AND PCDF CONGENERS IN FOOD OF ANIMAL ORIGIN IN ITALY: ASSESSMENT OF DIETARY INTAKE

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Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are ubiquitous toxic contaminants, highly lipophilic compounds that bioaccumulate in animal tissues. It is well established that food intake represents the main route of human exposure to these contaminants (more than 90%). Periodic surveys of the food supply for dioxins are a useful tool for monitoring changes in dioxin levels in the environment and to update predictions of human exposure to these compounds from dietary components. In the framework of the National Residues Surveillance Plan (NRSP), 543 food samples of animal origin such as meat, fat, milk and dairy products, eggs and fish, were analysed in Italy in the period 2000-2003 in order to assess PCDDs and PCDFs contamination levels.

The average dioxin daily intake through food of animal origin, based on mean value and 95th percentile, resulted in 0.33 and 0.82 pg World Health Organization-Toxic Equivalent/kg bw/day (WHO-TEQ/kg bw/day), respectively. Such values are below the 1-4 pg WHO-TEQ/kg bw/day tolerable daily intake recommended by the World Health Organisation. The highest contributions to dioxin daily dietary intake are due to fish (37%) and dairy products (28%), while the lowest one is given by poultry meat. Among examined matrices fish was the most contaminated (average of 5.16 pg WHO-TEQ/g fat content). The trout is the species that contributes more to this value, with an average of 5.90 pg WHO-TEQ/g fat. In the other matrices, such as meat, fat, eggs, milk and dairy products mean WHO-TEQ values are below 1 pg WHO-TEQ/g fat. Contamination profiles of various food products are presented. These are similar to those found in other European countries.

With a view to the inclusion of dioxin-like polychlorinated biphenyls (DL-PCBs) in the maximum dioxin levels in food set in the European Union, DL-PCBs have been included in the NRSP 2004. Data related to DL-PCBs levels in food are shown and their contribution to the overall dioxin intake is highlighted.

PROTEOMICS IN MONITORING FOOD QUALITY: EFFECTS OF AQUICULTURE ON THE SEA BASS (*DICENTRARCHUS LABRAX*) PROTEIN PROFILE

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Monitoring food quality is a primary task for analytical chemistry and represents a key step in assuring human health. Fish and fish products play an important role in human nutrition. Fish is a valuable source of highly assimilated proteins and it contains large amounts of PolyUnsaturated Fatty Acids (PUFA) and fat-soluble vitamins. However, as the world's wild fish stocks are limited, consumers are now being proposed farmed fish as an alternative. Moreover the fish muscle protein content has a great importance from an aquiculture perspective.

Much information on the physiology and biochemistry of fish muscle are reported; nevertheless, limited data on the changes of the protein pattern in fish due to different growth conditions are available for the time being. Application of proteomics in aquiculture may play a key role in the development of new breeding strategies and food sources. A proteomic approach based on SDS-PAGE separation of proteins, *in situ* protein hydrolysis, *de novo* sequencing of peptides by Liquid Chromatography-Mass Spectrometry-Mass Spectrometry (LC-MS-MS), protein identification and relative quantification of proteins by denaturing capillary electrophoresis was coupled with the determination by means of GasChromatography-Mass Spectrometry (GM-MS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) of fatty acids and metal ions content, respectively in farmed and wild sea bass.

The results have shown that aquiculture induces significant chemical and biochemical differences in fish muscle that may have an impact on the resulting food quality.

Poster session 2

Quality in analytical chemistry, food analysis

Chairperson
F. Cubadda

GAPS IN THE ANALYTICAL CHAIN FOR THE DETECTION OF GMOS IN SEEDS, FOOD AND FEEDS

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In the last decade a robust and reliable legislative package has been approved by the European Union in order to control the presence of GMOs in food and feeds and to gain consumer confidence. The reliability of the analytical chain influences many issues related to the above legislative package, representing a tool for labelling, traceability, coexistence and preventing the entering in the food chain of unauthorized GMOs.

At European level sampling and analytical methodologies have been recommended by the EU, both of them being rather reliable even though cost- and time-consuming. As for the analytical methodologies, PCR represents the basis for the detection of GMOs. However, several issues deserve further studies for the application to the enlarging variety of GMOs. In particular, difficulties are related to the modularity of the analytical procedure, to the measurements units (DNA-based or weight-based quantification), to the calculation of the uncertainty, to the CV of the methods, to the problem of stacked gene and to the biological factors. In addition, the strong need for (Certified) Reference Materials [(C)RMs] is hampered by several difficulties also related to the biological factors. Moreover, since other States, such as USA, are strongly pushing towards sampling and analytical methodologies (mainly protein-based methodology and seeds as the unit of measurement), substantially different from those adopted at European level, additional difficulties are related to the need to guarantee the compatibility between the two approaches. Conversion factors are therefore needed.

In all the above issues strong efforts have been devoted by the Community Reference Laboratory (CRL) acting within the Joint Research Centre (Ispra). In this work the CRL is assisted by the European enforcement laboratories.

PROFICIENCY TESTING SCHEMES ON ENVIRONMENTAL AND FOOD MATRICES: A PERMANENT COLLABORATIVE PROGRAMME OF INTERLABORATORY STUDIES

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The reliability and comparability of measurement data have been gaining increasing importance in the technological and scientific fields as well as in many other aspects of life due to the major role of science in modern society. The concept of quality has improved significantly during the last few years. Quality is frequently invoked when talking about productive processes. In chemical laboratories, the productive process is replaced by analytical processes where the product is information characterising the physical, chemical and/or compositional features of the analysed materials. Therefore, laboratory measurements and the obtained data also call for the implementation of tools assuring improved quality.

In order to control and/or assure the quality of laboratory procedures and data, the use of (Certified) Reference Materials [(C)RMs] as well as the participation in interlaboratory studies are of primary importance. The application of these tools allows measurement accuracy, reproducibility and long-term comparability of the data produced in the laboratory to be assessed. Participation in interlaboratory exercises and/or collaborative programmes (where the technical discussion of the results is included) is especially recommended (and in some European countries even mandatory) for obtaining and maintaining the accreditation status.

QualityConsult (QC) is an Italian non-profit association that has dedicated itself to the improvement of analytical quality. Since 2002 it has been actively involved in the field of quality control of analytical measurements. The activities of the QC involve the dissemination of knowledge on Quality Assurance/Quality Control tools and the promotion of their implementation in public, research and private laboratories through the organisation of Proficiency Testing Schemes, training courses and workshops. QualityConsult launched on a permanent collaborative programme of interlaboratory studies in 2004 in Italy by which it has been giving analytical laboratories the opportunity to participate in round robin tests on various sample matrices. The aim of the programme is to equip public as well as private laboratories all over Europe with tools for evaluating and improving the quality of their measurement data.

The first Interlaboratory Exercises, organised in the frame of the QC 2004 Programme, had over 50 participants, including laboratories from both the public and the private sector. A total of ten exercises were carried out throughout the year for the determination of trace elements, PCBs and PAHs in different environmental matrices (contaminated soil, sewage sludge and harbor sediment). Following the so-called ‘step-by-step’ BCR approach, the exercises addressed to the determination of trace elements in matrix RMs were coupled to a multielement/multicomponent synthetic solution. The use of this together with a selected matrix RM helped to verify the suitability of analytical methods and to identify possible problems that might occur at each individual step of the methods. In 2005 the collaborative programme will be extended outside Italy and include exercises both on environmental and on food matrices.

LONG-TERM STABILITY STUDY OF THE CANDIDATE REFERENCE MATERIAL *ADAMUSSIUM COLBECKI* FOR TRACE ELEMENTS

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In the framework of the Italian National Programme for Research in Antarctica (Programma Nazionale di Ricerca in Antartide, PNRA) a project was undertaken for the preparation and certification of new Certified Reference Materials (CRMs) for trace elements based on the Antarctic scallop *Adamussium colbecki*. After the between-bottle homogeneity and the short-term stability tests, the long-term stability study was carried out to evaluate the effect of long-term storage at a given storage temperature on the material under investigation.

The trace elements taken into account for the *Adamussium colbecki* candidate CRM were As, Cd, Cr, Cu, Fe, Mn, Ni and Zn. For the above-mentioned test, the determination of the elements of interest was performed on samples mineralised by microwave-assisted acid digestion. The analytical techniques employed to this end were Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and Dynamic Reaction Cell Quadrupole Inductively Coupled Plasma Mass Spectrometry (DRC-Q-ICP-MS). These analytical techniques were chosen on the basis of the trace element concentrations expected and of the anticipated spectral and mass interferences so as to minimise these last to the largest possible extent. The outcome of the long-term stability study was entirely satisfactory.

In consideration of this, the certification campaign can be actually undertaken. To this end, a number of laboratories from various countries with experience in trace element determinations in biological matrices have been already selected, to which a number of vials of the *Adamussium colbecki* candidate CRM will be distributed. All participants will have to carry out the element determinations by instrumental techniques of their choice.

DETERMINATION OF ESSENTIAL AND TOXIC TRACE ELEMENTS IN ITALIAN AND ASIAN RICES BY ICP-AES AND ICP-MS

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Rice is one of the major sources of food for a large part of the human population, especially in Asian countries. In this regard, potentially toxic elements contained in rice cultivated in contaminated groundwater for irrigation can pose significant risk to the health of the consumer. In particular, several studies reported on the ability of rice to accumulate As. The purpose of this study was to compare the contents of As as well as of Cd, Co, Cu, Fe, Hg, Mn and Zn in different types of rice grown in Asia with those of Italian rice. Plasma-based techniques were resorted to to carry out this study, namely, Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and Dynamic Reaction Cell Quadrupole Inductively Coupled Plasma Mass Spectrometry (DRC-Q-ICP-MS). In particular, As, Cd, Co and Hg were quantified by DRC-Q-ICP-MS and Fe, Mn and Zn by ICP-AES. The accuracy of the whole procedure was tested by using the Certified Reference Material (CRM) NIST 1568a Rice Flour. Arsenic was found to range from 69-208 ng g^{-1} for Asian rice and from 126-202 ng g^{-1} for Italian rice.

ORGANOTIN DETERMINATION IN FRENCH DRINKABLE ALCOHOLS BY HEAD-SPACE SOLID PHASE MICRO-EXTRACTION GAS CHROMATOGRAPHY PULSED FLAME PHOTOMETRIC DETECTION

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Organotins are normally used for agricultural and industrial applications due to their biocide and fungicide properties. However, these compounds are potentially toxic, the trisubstituted ones being responsible for a significant number of ecological disasters. Tributyltin, in turn, is now recognised as an endocrine disrupter. Nowadays, the risks induced by organotin accumulation in human body are not clearly identified, but they are suspected to be hazardous for human health. Moreover, they are often found in foodstuffs by direct or indirect supply. Having regards to these data, a quantification of organotins in everyday life products and foodstuffs is essential to assess the daily human exposure to these toxic compounds. This study deals with the development of an analytical method to quantify organotin compounds in French drinkable alcohols (liqueur wines, French brandies and spirits).

The analysis is based on the aqueous ethylation using Na tetraethylborate ($NaBEt_4$) as a derivatisation agent, separation by Gas Chromatography (GC) and specific detection, using Pulsed Flame Photometric Detection (PFPD). This process has been previously validated for environmental analysis. Along with derivatisation, the analytes are collected by Head-Space Solid Phase Micro-Extraction (HS-SPME). This procedure is preferred to the usual Liquid Liquid Extraction (LLE) to avoid matrix effects and improve sensitivity. The HS-SPME is performed with either a 100 µm thickness polydimetylsiloxane (PDMS) film or a 75 µm thickness carboxen/polydimethylsiloxane (CAR/PDMS) film. The two fibres were previously found to be convenient for environmental organotin speciation.

The optimisation of the SPME operating conditions and the analytical performance are described. Applications to various alcohol samples are also presented.

DETERMINATION OF SEMICARBAZIDE IN BABY FOOD: A EUROPEAN SURVEY

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To date, semicarbazide (SEM) is determined in food as a marker to detect the illegal use of the banned antibiotic nitrofurazone. However, it was recently found that this substance is also present in certain foods packed in glass jars and bottles closed with metal lids sealed with plastic gaskets that are foamed using the chemical blowing agent azodicarbonamide (ADC). The food commodities concerned included fruit juices, jams and preserves, honey, baby food, pickles and sterilised vegetables, mayonnaise, mustard, sauces and ketchup. Azodicarbonamide is also used as an additive for flour (due to its dough-improving properties) in some countries such as Canada, USA and Brazil. In the European Union the use of ADC as flour additive is banned. Recent studies proved that SEM can also be formed in processed food, such as coated poultry products and in bread prepared with ADC-containing flour. Formation of SEM in processed food samples, such as carrageenan-containing food, treated with hypochlorite for disinfection and bleaching has also been reported.

Of particular concern is the presence of semicarbazide in baby food because the intake/body weight ratio is higher than for adults and because some babys, aged between 6 and 12 months, eat almost exclusively food packed in glass jars. In the present work a survey was made of the content of SEM in baby food. About 100 samples bought in supermarkets in 13 countries of the European Union have been analysed. A Liquid Chromatography-Mass Spectrometry-Mass Spectrometry (LC-MS-MS) method validated in a collaborative study has been used for the determination of SEM. The statistical evaluation of the results is also illustrated.

EVALUATION OF THE ANTIOXIDANT ACTIVITY OF SOME OLIVE OIL POLYPHENOLS BY MEANS OF AN ELECTROCHEMICAL METHOD

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Oxidative stability is a central parameter in the estimation of Extra-Virgin Olive Oil (EVOO) quality, as it gives a reliable evaluation of the susceptibility to oxidative degeneration, which is the main cause of its adulteration. The shelf-life of olive oil is strictly related to rancidity development, which could depend on the autoxidation of fatty acids. This phenomenon takes place in the presence of oxygen, generating some unstable compounds that can modify the sensory and nutritional characteristics of the oil, thus leading to product spoilage.

Although inevitable, the oxidation process can be delayed by endogenous antioxidant that enhance the oxidative stability by preventing the propagation of lipid peroxidation or removing free radicals. Antioxidants are reported as molecules which, when present at low concentrations compared to those of an oxidable substrate, significantly delay or prevent oxidation of that substrate. A classification of the antioxidants can be established based on their mechanism of action as follow: *i*) primary antioxidants, that terminate the oxidation chain reaction by donating hydrogen or electrons to free radicals; *ii*) synergistic antioxidants, that are classified as oxygen scavengers and chelators; and *iii*) secondary antioxidants, that prevent the oxidation by decomposing the lipid peroxides into stable end products. An important aspect of the study of antioxidants is the assessment of antioxidant activity. Various methods have been introduced to test antioxidant activity of olive oil. Most of them investigate the ability of oil to scavenge a free radical, while few tests are based on the study of electrochemical properties by voltammetry or amperometry.

In the present study, some polyphenol of EVOO, such as simple phenols, lignans and secoiridoids, obtained by High Performance Liquid Chromatography (HPLC) fractionation were evaluated with respect to their antioxidant activity by chemical methods (DPPH and ABTS) and an electrochemical method. The aim of the work was to understand the antioxidant activity of different molecules and compare the information obtained with the various analytical methods in order to have an insight of the chemical groups involved in the antioxidant mechanisms.

RAPID ELECTROCHEMICAL DETERMINATION OF LACTOPEROXIDASE IN RAW MILK

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Somatic cell count is a important parameter for the characteristics of bovine milk. The bovine milk defenses against bacterial infections can be separated into two distinct categories, *i.e.*, cellular and soluble defenses. The latter work independently and together with antibodies and cellular factors to provide protection to the mammary glands. These include lactoferrin, complement, lysozyme and the lactoperoxidase-thiocyanate-hydrogen peroxide system (LPS). Lactoperoxidase (LP) is produced in small amount by mammary gland. The levels of thiocyanate in the mammary gland are dependent on the nutrition schedule. Bovine LPO is a protein of 612 amino acids with a molecular mass of approximately 78,000 Da, *ca.* 10% of which is carbohydrate. The rapid detection of LPO activity on site could then represent a valuable tool for the assessment of quality of raw milk .

In this work the development of electrochemical methods based on flow injection analysis for the determination of LP in raw milk is reported. The peroxidative mechanism for LPO, as it is true for all hemoprotein peroxidases, involves the following three-steps sequence, where AH is a substrate.

$\text{LPO} + H_2O_2 \rightarrow \text{Compound I} + H_2O$ (Eqn. 1)
$\text{Compound I} + \text{AH} \rightarrow \text{Compound II} + \text{A.}$ (Eqn. 2)
$\text{Compound II} + \text{AH} + \text{LPO} + \text{A.} + H_2O$ (Eqn. 3)

The method is based on the use of an electroactive co-substrate of LP such as tetramethylbenzidine (TMB) or ferrocene carboxylic acid (FCA). The assay has been carried out in an electrochemical cell with a glassy carbon working electrode. Experimental parameters, such as substrate/co-substrate concentration, flow rate, dilution loop and pre-incubation time, were optimised in order to obtain a linear calibration curve in a useful working range (1-10 U/ml). Problems arising from matrix components, mainly lipids, on the measuring system were also evaluated and are reported and discussed.

Oral session 6

Environmental analysis (general aspects)

Chairperson
R. Morabito

METHODOLOGICAL APPROACH TO EVALUATE THE ENVIRONMENTAL IMPACT OF OIL AND GAS OFFSHORE PLATFORMS ACTIVITIES

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In order to assess the potential environmental impact due to the installation and activities of offshore oil and gas platforms, the Central Institute for Marine Research (ICRAM in the Italian acronym) has drawn up a methodological approach encompassing multidisciplinary investigations. This approach allows for the assessment of the impact (biotic and abiotic environmental alterations) resulting from the different phases of offshore activities (exploration, production and decommissioning). Various investigations, such as chemical and physical analyses of water and sediment, benthic fauna analysis, bioaccumulation testing, fish stock assessment and indirect acoustic investigations were carried out to evaluate the potential impact caused by the installation of the offshore platforms.

Moreover, ICRAM has drawn up a specific monitoring plan to evaluate the environmental impact of produced water released at sea and in particular the concentration and spatial distribution of selected compounds commonly present in the produced water, such as trace metals, organic compounds and BTX and their bioaccumulation in mussels living on the installation piles. Additionally, in order to evaluate the ecotoxicological effects of the produced water, a battery of bioassays were applied to the produced water, seawater and pore water extracted from sediments.

The methodological approach used to study the different potential impact of the offshore activities on the marine environment is illustrated along with some important results of the monitoring activities that ICRAM has carried out since 2000.

CHEMICAL AND BIOLOGICAL INFORMATION STORED BY FRESHWATER BIOFILMS

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Biofilms are formed on the surface of artificial or natural substrates located in the photolytic layer of surface waters. The main components of biofilms are the bacteria, algae and their metabolic products (*e.g.*, polysaccharides, lipids, proteins *etc.*). These last compounds belong to the so-called Extracellular Polymeric Substance (EPS) matrix. Due to the large number of functional groups (carboxyl, hydroxyl *etc.*) the EPS-matrix has an excellent chelate-forming ability. Metal ions and different organic residues (pesticides, herbicides) can be bounded to the biofilms. It should be emphasized, however, that not only the EPS-matrix, but also the micro-organisms are able to accumulate various contaminants from the surface waters. The enrichment factors for different compounds amount to 103-104.

In order to use the biofilms for monitoring water quality of lakes or rivers, it is necessary to understand their formation, the colonisation processes of micro-organisms and to know exactly which properties of the substrates can influence the colonisation and the biomass production of the micro-organisms. From the biological point of view the biofilms formation is successful if the micro-organisms living in the surface water are represented in large number in the biofilm. In this case the algological and bacteriological investigations result in reliable biological information about the possible ecological changes. Certainly the chemical investigations of biofilms help finding the critical impurities, applying modern analytical techniques. For trace metal determination total-reflection X-Ray Fluorescence Spectrometry (XRFS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) are recommended due to their excellent analytical capabilities (low detection limits, multielemental detection). Organic contaminants need more sophisticated analytical procedures, especially because their separation from the matrix is a critical step. Their determination can be carried out by Gas Chromatography Mass Spectrometry or Liquid Chromatography Mass Spectrometry systems.

In the lecture the following experiments are presented and discussed: *i*) colonisation of micro-organisms on polycarbonate film during the first 126 hours; *ii*) formation of biofilms in freshwater lakes in Turkey and Hungary, as well as in the Tisza river; *iii*) effects of Ni and Pb contamination on the biological and chemical composition of biofilms.

THE USE OF BENTHIC FORAMINIFERA TESTS AS PROXY FOR DISSOLVED METALS IN SEAWATER, HAIFA BAY, ISRAEL

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The Mediterranean coastal waters of Israel are exposed to heavy metals introduced from point (marine outfalls, coastal rivers) and diffusive (runoff, atmosphere) sources. As a result, a number of local pollution sources exist along the coast, especially at the southern part of the Haifa Bay. However, the dissolved heavy metal concentrations in the bay and in the shallow water along the coast are poorly known. The determination of heavy metal concentrations in seawater, even with sophisticated instrumentation such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS), is analytically challenging due to their extremely low concentrations (ng/l) and their high dissolved solids matrix (up to 4.0%). The analysis requires separation and pre-concentration of heavy metals on ion exchange resin columns in a clean room environment. Because of this analytical complexity it is difficult to establish a routine monitoring programme and assess the water quality in terms of ecological environmental criteria.

The purpose of this study was to examine the use of metal concentrations in benthic foraminifera tests (*Amphistegina lobifera*) as proxy for dissolved metal concentrations in seawater. The tests precipitated by *A. lobifera* establish an equilibrium state with the ambient seawater represented by a metal explicit distribution coefficient. These foraminiferal tests integrate the heavy metal seawater concentration during their lifetime that varies seasonally from 4 to 12 months. The analysis of foraminiferal tests (following a cleaning procedure that removes detritus and, primarily, the inner cytoplasm) is relatively quick and simple.

The foraminifera *A. lobifera* is a large epiphyte very abundant in the Haifa Bay in large numbers on hardgrounds (calcareous rocks) in ~10 m water depth. It was sampled bi-monthly in addition to surface seawater at six representative stations in Haifa Bay, in eight cruises during 2003-04. The spatially composite seawater samples were slightly contaminated by heavy metals compared with the open Mediterranean water sampled 20 km offshore or with coastal water from the northern Gulf of Aqaba (Red Sea). The foraminiferal test composition show that all suites of dissolved metals were easily detected in their tests. Temporal variations in tests composition indicate a characteristic behavior of some groups of metals of common source or effects of calcification rates. Based on seawater – foraminifera analysis it seems that Cd, Cu and Zn are the most enriched metals in the Haifa Bay. However, further research is needed to better understand the inter-relationships between seawater and foraminifera tests chemical composition.

APPLICATION OF SEDIMENTARY BIOMARKERS AND STABLE ISOTOPES IN PALEOENVIRONMENTAL RECONSTRUCTIONS

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Various biomarkers, such as lipids, pigments, lignins and also stable carbon and nitrogen isotopes provide useful information about the sources and historical changes in organic matter, as well as changes in the trophic status of lakes. Lipids, *i.e.*, aliphatic hydrocarbons, aliphatic alcohols, sterols and fatty acids, as well as stable carbon and nitrogen isotopes were determined in the sediment of Jezero na Planini pri Jezeru, a small, remote mountain lake (NW Slovenia). The trophic status of the lake has changed over the past decades and the lake is currently eutrophic. It was found that short chain lipids, that are mostly contributed by plankton and bacteria, predominate in the surface sediment layer due to high primary production in the lake. In contrast, longer chain lipids were the most abundant constituents in the deeper sediments. These homologues are mainly of terrestrial origin. Their enrichment with depth can be explained by the selective degradation of lipids, since longer chain homologues are less susceptible to degradation. In addition, evident changes in the $\delta\ ^{13}C_{org}$ profile were observed in the sediment, also suggesting that sources of organic matter and the trophic status of the lake have changed. In contrast, $\delta\ ^{15}N$ values were quite uniform with depth, suggesting that sources of N have not changed considerably. Thus, molecular and isotopic analysis in sediments can successfully be applied in paleoenvironmental reconstructions to follow changes and processes in the lake and its watershed that are either natural or anthropogenically caused.

FISH DIET AS INTEGRATED TOOL IN ECOLOGICAL ANALYSIS ON LARGE RIVERS

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Recently, the increasing need for an integrated approach in environmental assessment has been stressed. This is of particular relevance in the ecological assessment of large riverine ecosystems, where the sampling difficulties in the characterisation of the complex of different microhabitats can affect both the classification and the evaluation procedure. In this framework, fish feeding habit might be seen as a sampling gear of lower level biotic taxa (*i.e.*, benthic taxa). This study is part of a comprehensive ongoing investigation on the fish fauna and lotic ecosystems of the low reach of Tiber river. The river ecological status has been evaluated, in a holistic way, through an integrated approach based on fish assemblage assessment, chemical and microbial analysis, benthic communities used in biomonitoring and river ecosystem's functions analysis.

The diet composition of *Barbus tyberinus*, *Leuciscus cephalus*, *Rutilus rubilio*, *Pseudorasbora parva*, *Ictalurus melas* from the Tiber river (central Italy) was investigated. Water quality was assessed by the analysis of chemicals, biological (IBE index), microbial and ecosystemic (IFF) parameters. Both interspecific and intraspecific differences in fish diet were revealed by means of univariate and multivariate statistical methods. Information about benthic community structure obtained from fish diet analyses and from conventional sampling has been compared in order to point out differences between these approaches. The analysis of omnivorous fish feeding habits seems to have a potential as a proxy for an optimal sampling procedure aimed at benthic macroinvertebrates, which is very difficult to be arranged in large rivers because of spatial complexity and a number of operational constraints affecting conventional sampling strategies.

THE ANALYSIS OF FISH PARASITES AS A COMPLEMENTARY TOOL IN MONITORING OF RIVERINE ECOSYSTEMS

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In the last years, there has been increasing experimental evidence about the feasibility of using fish parasites as bioindicators of the environmental stress. Parasites could be a good indicator of environmental conditions because of their complex life cycle that can involve several animal species belonging to different taxa and complex biocenotic interactions. Moreover, recent studies have revealed the possibility to use some taxa, *e.g.*, acanthocephalans and cestodes, as indicators of metal pollution since the adults of these parasites can accumulate heavy metals at concentrations orders of magnitude higher than their host. The present research is part of a comprehensive ongoing investigation of the fish fauna and the lotic ecosystem of the low reach of the river Tiber, the major river of Central Italy in terms of basin area and length. During this investigation, a characterisation of the environmental status was performed through physical, chemical and microbiological analysis. In particular, the aim of the present study was the characterisation of the helminth fauna of chub *Leuciscus cephalus*, *L.*, from the area under investigation. The data of the parasitological analysis were correlated with the features of the habitat. Results show a close correlation between the helminth composition and the environmental deterioration and confirm the possibility that the changes in parasite communities between different sites could be used to clarify the environmental status in combination to chemical and microbiological analysis.

APPLICATION OF SELECTIVE MEMBRANE PRECONCENTRATION TECHNIQUE FOR REAL SAMPLE ANALYSIS BY ARTIFICIAL OLFACTORY SYSTEMS

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There are two major problems with the analysis of samples from the environmental, food and medical fields in terms of their composition by Artificial Olfactory Systems: the low concentration of the analytes and the presence of a bulk interferent. Low concentrations can be circumvented by introducing an appropriate sample preconcentration, while the interferent – commonly water (humidity) and ethanol – may be removed at the same time or in a further separation step. A compromise frequently needs to be found between the resulting time for overall sample analysis and quality of the analysis itself. Both preconcentration and interferent removal techniques are usually based on physical-chemical principles and therefore rarely enrich or deplete exclusively the target compounds. Hence, sample work-up commonly goes along with a loss of complexity of the original sample. This work proposes a membrane separation technique, pervaporation, as a means for selectively enriching analytes with regard to interferents in one separation step and without losing any of the original sample constituents. The technique operates at ambient temperature, without the need for an extraction aid other than the membrane, which in turn does not in principle suffer any structural alterations with increasing sample throughput.

Two case studies were undertaken in order to study the feasibility of pervaporation as an analytical sample work-up technique for analysis with “electronic noses”, namely the analysis of wine aroma compounds and the analysis of human breath. The high ethanol content of the former and the humidity of the latter impede the detection of the target compounds for classification. Samples from both case-studies are furthermore highly diluted and thus require the analytes to be concentrated prior to analysis. Pervaporation was employed using a non-porous hydrophobic membrane based on polydimethylsiloxane and the separation performance monitored by both an “electronic nose” based on metal-oxide semiconductors, and a Gas Chromatography Mass Spectrometry (GC-MS) for validation. A semi-automated integrated pervaporation-electronic nose system was set up and its feasibility demonstrated with regard to the diminution of the effect of the interferent, the detection of the target analytes, as well as the overall analysis time. This work will show that pervaporation is a versatile and simple technique which may be tailor-made for individual applications. Finally, pervaporation is put into context with conventional preconcentration techniques, and its potential and limits are highlighted.

OLFACTORY ANNOYANCE ASSESSMENT OF ODOROUS ENVIRONMENTAL POLLUTANTS BY DYNAMIC OLFACTOMETRY

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Assessment of olfactory annoyance is extremely difficult to achieve through the use of analytical techniques. This is due to the low concentrations of the most important components of odours, the complexity of these gaseous mixtures that may consist of hundreds of compounds, and the unpredictability of human perception due to the high non-linearity of the olfactory system. These reasons along with the ever growing public demand for protection against this kind of pollution have made olfactometry more and more interesting. In fact, this psychophysical technique was recently codified by the European technical guidelines EN 13725. Olfactometry is the controlled presentation of odorants as well as the registration and analysis of the resulting sensations in man. It is an effect-related measuring method which has two objectives: firstly, to determine unknown odorant concentrations with the help of the human sense of smell as the detector and secondly to determine the power of the human sense of smell, with known odorant concentrations serving as defined stimulus intensities and thus as scale increments. Other issues can also be addressed by olfactometry (VDI 3882) such as the determination of the intensity of the stimulus and the hedonic tone, which provide indications of the likely offensiveness of odours.

In the present work a waste treatment plant is considered that is capable of processing about 150,000 tons/year of urban and industrial solid wastes with an exhaust air cleaning system consisting of two scrubbers and a six-bed biofilter. Air was emitted in the atmosphere through a chimney at an average speed of about 11 m/sec. As a result of the optimisation of the air sampling procedure, Nalophane™ bags (50 l) were found to be the best option and were used to collect samples according to a method based on the lung principle. Samples were collected in the waste treatment plant and were analysed by dynamic olfactometry with an eight-person panel within 24 hours after sampling. Each sample underwent three series of measurements to assess the individual threshold (the first series was excluded from data processing), in accordance with the above mentioned European guidelines. Odour concentration was 550 OU/m^3 at the chimney and 6120 OU/m^3 upstream of the biofilter, which corresponds to a 91% abatement efficiency of the odour level. Finally, the olfactory impact in the area surrounding the plant was assessed by using atmospheric dispersion models based on orographic and meteorological data. The latter were supplied as hourly averages data for the period 1998-2002 by two regional agencies, namely ARSIA and ARPAT. Long- and short-term simulations showed that the biofilter almost cancels the odour impact of the waste processing plant.

PALLADIUM SUPPORTED ON TETRAGONAL ZIRCONIA: ELECTROSYNTHESIS, ANALYTICAL CHARACTERISATION AND APPLICATION IN THE ABATEMENT OF POLLUTANT EMISSION IN THE ATMOSPHERE

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Core-shell Pd nanoparticles were supported on ZrO_2 nanograins *via* a sacrificial anode electrosynthesis route. Several samples were prepared varying the electrolysis time and thus the Pd loading in the nanocomposite. X-Ray Diffraction (XRD) analysis showed that ZrO_2 was mainly present in the tetragonal phase. Surface areas measured by the BET method were comprised between 70 and 80 $m^2\ g^{-1}$. Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) was used for the analysis of the nanopowders bulk composition, this being in the range of 0.9-2.7%, as function of the electrosynthesis conditions.

X-ray Photoelectron Spectroscopy (XPS) analysis allowed the surface elemental composition of the nano-materials to be quantified and the different chemical states of the nano-dispersed Pd to be studied. The surface composition mirrored that expected on the basis of the electrochemical (coulometric) and ICP-AES data. The study of high resolution Pd3d XP spectra revealed that nano-sized Pd is mainly present at the zero oxidation state, while Pd(II) compounds can be found as a minor chemical component.

Transmission Electron Microscopy (TEM) revealed that the Pd-ZrO_2 nano-composite powders are composed of spherical Pd nano-particles evenly dispersed on the surface of the ZrO_2 grains. The average core diameter of the catalyst inclusions is 6.9±1.8 nm, while the zirconia average grain size is 150±70 nm.

Catalysts were tested for CH_4 total combustion and for CO oxidation. The conversion increased with Pd content in the CH_4 combustion experiments, whereas it decreased with Pd content in the CO oxidation experiments. The apparent activation energies were about 25 kcal mol^{-1} and 9 kcal mol^{-1} for CH_4 combustion and CO oxidation, respectively. It is assumed that different reaction mechanisms and different active sites are probably involved in the two reactions.

Oral session 7

Environmental analysis (specific aspects)

Chairperson
R. Fuoco

RECENT DECREASE OF Pb IN THE ENVIRONMENT: AFTER ANTARCTICA, THE SNOW OF APENNINES AND THE ADRIATIC SEA

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Ten years ago, after a similar observation had been reported for the North Pole, it was noticed that the content of Pb in Antarctic snow had been decreasing since the mid-eighties due to the banning of this metal from gasoline in the countries of the southern hemisphere. Following this discovery a research programme on the Pb content in the Italian environment started in the year 2000 in parallel with the process of removal of the metal from gasoline, which has been totally Pb-free since January 2002. So far, studies have been carried out on the snow of the Apennines and the seawater of the northern Adriatic Sea (coastal and open sea).

Each year, during the winter, samples of fresh snow from the summit of Mount Cornaccione (Frontignano, Marche) at an elevation of about 1700 m are collected. As regards seawater, both coastal and open sea environments were studied. Seawater was collected systematically at three sites along the coast line near the city of Ancona (the mouth of the River Esino, an area close to the API refinery and Portonovo beach, a tourist zone of the Conero mountain coast), each other week initially, then weekly and now monthly. Samples from the open sea have been collected over these last three years during three oceanographic cruises in the northern Adriatic Sea carried out to study seawater in a transect from the mouth of the River Po to the centre of the Adriatic Sea.

The samples collected were analysed by Square Wave Anodic Stripping Voltammetry (SWASV), which gave simultaneous determinations of Cd, Cu and Pb. The results for Pb were particularly interesting from an environmental viewpoint. All results show that over the last few years environmental Pb has greatly decreased. In particular, the results obtained on snow show that the Pb concentration passed from thousands of pg/g to hundreds of pg/g. In coastal seawater soluble Pb concentration diminished from an average value of 77 ng/l in 2001 to about 46 ng/l in 2002 and to about 31 ng/l in 2003. In the open sea Pb diminished by about 80% just in the period between autumn 2002 and summer 2003. Preliminary results for samples collected in spring 2004 confirm the results obtained in summer 2003. The results obtained are examined and compared with the statistical data for the reduction of Pb emission into the atmosphere in Italy over the last five years.

INFLUENCE OF Fe-Mn OXIDES AND ORGANIC MATTER ON METAL SEDIMENTATION IN NORTHERN ADRIATIC SEA

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Metal can be scavenged from surface waters to surface sediments by Mn(III/IV) and Fe(III) oxide/hydroxides or by organic ligands which form stable complexes with heavy metals. The knowledge of their total concentration in sediments is important in order to collect information on marine contamination, whereas metal speciation allow the metal availability and the toxicity of sediments to be predicted. The total metal concentrations of Cu, Ni, Pb and Zn were studied along with their distribution in different chemical forms in order to know the extent of sediment contamination and to evaluate the bioavailability of metals. Concentrations of iron, Mn, Total Organic Carbon (TOC), humic acids and fulvic acids were also considered.

The study was carried out on marine sediments sampled in two areas of the Adriatic Sea that could be influenced in different way by the Po river waters. In the first zone a salinity gradient from the coast (34.2 PSU) to open sea (38.0 PSU) was observed, indicating the influence of fresh water from the river Po, whereas in the second zone salinity values ranged from 36 PSU to 38 PSU. The distribution order of metals in the sediments was Fe > Mn >Zn > Ni > Pb > Cu.

Significant correlations between total concentrations of Cu and Zn and between Ni and Pb, respectively, suggest common and specific sources, whereas the accumulation of metals in the sediments appear to depend prevalently on non-oxidic sorbent phases, such as organic matter. In the sediments the distribution of Cu, Fe, Mn, Ni, Pb and Zn in the different chemical forms depends on the nature of the metal as a results of the different degree of affinity for organic and inorganic compounds. In almost all samples the prevailing Zn phases are mobile forms and sulphide. Nickel is present in high percentage in sediments as sulphide, whereas the preferential association of Cu with organic matter is evident. Manganese and Pb are preferentially present as mobile forms.

The high Mn concentration in a mobile form may be the consequence of the metal dissolution under anoxic conditions. The relation between Pb present in mobile forms and the redox potential values ($r^2 = 0.78$) might be in favour of the recycling process of the metal. The high percentage of Pb in mobile forms together with the fraction of the metal sorbed on oxidic material (step B) make the sediments examined potentially dangerous.

ORGANOTIN COMPOUNDS IN SEWAGE SLUDGE FROM A WASTEWATER TREATMENT PLANT

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Organotin Compounds (OTCs) are highly toxic substances. The European Community has included some of them into the list of priority pollutants. Significant efforts have been made in order to propose standardised analytical methods for water, sediment or soil quality control. In addition, environmental monitoring is also required especially in freshwater systems, including treatment plants, where OTCs are systematically present due to their wide household use.

Development and validation of a method for the speciation of OTCs in sewage sludge samples were the first two aims of the present study. The method is based on acetic acid extraction followed by derivatisation with Na tetraethyl borate. The ethylated species are extracted into *iso*-octane and their concentrations determined by Gas Chromatography Pulsed Flame Photometric Detection (GC-PFPD). Since there are no Certified Reference Materials (CRMs) available for sewage sludge, the accuracy of the analytical procedure was checked by the analysis of the CRM PACS-2 (marine sediment). Good agreement between experimental and certified values was obtained for butyltin compounds.

The second goal was to estimate the level of OTCs in sewage sludge from Slovenian waste water treatment plant. Interferences from sample matrix, arising presumably from S species present in the sewage sludge were observed. Therefore, the detector operating conditions have been studied and optimised. A particular interest was given, for the first time, to the discontinuous detection, which is tuned to the pulsed flame frequency. The photometric emission of the combustion products was especially considered due to the high noise of the baseline. This leads to a particular gate delay and gate width adjustment. Another crucial analytical aspect was the representativity of sewage sludge sample. Homogeneity of the sewage sludge was tested by the analysis of 10 individual aliquots using 20 and 100 cm^3 of acetic acid buffer solution. The sludge contained butyl- and octyltins, with concentrations ranging from 30 to 200 ng g^{-1} and exhibiting a moderate contamination. The results indicated that there was no significant difference between data obtained by applying 100 or 20 cm^3 of acetic acid buffer in the derivatisation-extraction procedure. Analysis of 10 individual samples showed good agreement of results for OTCs. The differences in general did not exceed 15%, thus providing evidence for good homogeneity of the sewage sludge sample.

CREMATORIA AND URBAN WASTE INCINERATORS Hg EMISSIONS

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Mercury has been recognised as an element with adverse effects on the environment and human health. Solid scientific evidence has justified the need for international agreements on Hg emission reduction. These agreements and strategies or their implementation are now available within the EU Directives and the European marine conventions. This study reports on Hg emission from some operating waste incinerators and crematoria in Italy. Waste incinerators and crematoria may represent a considerable source of Hg emissions. Recently adopted national waste strategies may lead to the increase in the number of incinerators in Italy. The number of cremators will also grow in Italy owing to such factors as the current increasing demand for cremation as a system of burial and as a system of disposal of non-decomposed corpses, resulting from operations of exhumation.

Data from some operating waste incinerators emissions in Italy, gave the following Hg emission factors: 0.30÷51 g per ton of waste before the flue gas cleaning systems (on untreated flow); 0.01÷1 g per ton of waste after the flue gas cleaning systems (treated flow). Data on Hg emission from some cremators range from less then 0.02 to 0.3 mg/Nm^3 (calculation basis: 3500 Nm^3 air per cremation, 55 cremations, for a cremation process lasting 2 h) and the range of the emission factor is 0.07 to 1.05 g/body. The measured levels of Hg vary between tests: this may be attributable to the presence or not of dental amalgams in the teeth of the deceased.

As a matter of fact, the practice of cremation is not very much used in Italy. Hence, there is great uncertanity in the calculated amount of Hg. Morever, dental amalgam fillings are responsible for Hg release upon cremating and the average of Hg per each body prior to cremation is unknown. Although emissions from incineration and cremation facilities can be smaller than emissions from other types of sources, it is important to assess incinerator emissions in the context of the total ambient concentration of pollutants in a given area. In areas where the ambient concentrations are already close to or above environmental guidelines or standards, even relatively small increments can be important.

BIOMIMETIC RECEPTORS FOR PESTICIDES

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The idea at the base of this study was to develop bio-mimicking receptors to be incorporated in affinity media for compounds of evident toxicity like pesticides. Strategies based on oligopeptides, which work mimicking the biological binding site, can overcome the disadvantages of biological materials (unstable, expensive to produce *etc*.). To this end, a computational and experimental approach has been used.

In computational screening information available on the natural biological receptor, the target enzyme of pesticides, acetylcholinesterase (AChE), has been used as a guideline to reduce the number of structures to be computationally tested. This has consequently resulted in small libraries of oligopeptides (tetrapeptides). Then a series of receptors based on aminoacids motif has been designed and their affinity toward organophosphate (OP) and carbamate (CM) pesticides has been tested by a computational approach using Leapfrog® algorithm, a module from Sybyl Sofware. The estimated computational binding energy of the selected oligopeptides toward a virtual array of pesticides has been computationally obtained.

Experimentally, different sequences of tetrapeptides virtually able to bind the two classes of pesticides were synthesised and tested using spectroscopic study. The UV-visible studies revealed that the receptors bind to pesticides to give a supramolecular complex by noncovalent interaction. In particular, the changes observed allowed the association constants at micromolar concentrations to be calculated. The selectivity against pesticides was nicely correlated to the estimated binding energy of the receptors calculated by computational modelling.

As a practical analytical application three tetrapeptides, with different binding energy *vs.* pesticides, were selected and their affinity towards two different pesticides was tested using an electrochemical biosensor for AChE inhibitors. Concentrations, incubation time and ionic strength of the oligopeptide-pesticide solution were evaluated using this electrochemical screening method. These data, together with the preliminary data obtained using solid phase extraction columns modified with the selected tetrapeptides, are presented and discussed.

Oral session 8

Pharmaceutical and clinical analysis

Chairperson
S. Caroli

A NEW RELIABLE METHOD FOR HPLC-UV SIMULTANEOUS DETERMINATION OF 5- FLUORURACIL AND METHOTREXATE

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5-Fluoruracil (5-FU) and methotrexate (MTX) are the most widely used antineoplastic drugs in therapeutic protocols for treatment of cancer. These drugs may expose hospital personnel and health care workers to high environmental levels. The principal route of exposure is inhalation of aerosol by means of drug dusts or droplets, dermal adsorption and accidental ingestion by means of contaminated foods. Although there is insufficient information about the long-term effect of occupational exposure to cytostatic drugs (CDs) and related wastes, there is clear evidence pointing to adverse health effects like contact dermatitis, alteration in normal blood cells count and liver damage in occupational exposure. Hence, even small quantities of CDs should be avoided as much as possible in order to reduce occupational exposure to levels compliant with the as low as reasonably achievable (ALARA) principle.

The aim of this work was to develop a simple chromatographic method for the simultaneous determination of both analytes in matrices such as wipes and pads. Separation of 5-FU and MTX was accomplished on a reversed-phase column system composed of a Nucleosil C18 analytical column using an elution gradient of about 20 min of ammonium acetate solution at pH = 4, with water and methanol at a flow of 1 ml/min and with a hediode array detector set at two wavelengths (260 nm for 5-FU and 310 nm for MTX). Recovery studies, performed by preparing 5 standard solutions (1, 0.5, 0.2, 0.1 and 0.05 μg g^{-1}) of MTX and 5-FU, were carried out in triplicate. Recoveries obtained for MTX ranged from 94 to 105%, whereas for 5-FU ranged from 95 to 106%.

This method could be a useful tool for checking the efficacy of prevention and protection measures. In fact, analyses should be carried out on samples collected from various surfaces inside laboratories where CDs are handled. Data obtained from those samples could provide a valuable information on the potential exposure of health care workers *via* skin contact with contaminated surfaces.

PVC AND SOL-GEL SENSOR MEMBRANES BASED ON Mn(III) TPP-CL FOR THE DETERMINATION OF VALPROATE IN PHARMACEUTICAL PREPARATIONS

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Metalloporphyrins have emerged as promising sensing species for the preparation of potentiometric units that enable the direct determination of specific analytes with better characteristics than those based on quaternarium ammonium salts. On the other hand, the research of new materials is important for the construction of ion-selective electrodes which can improve electrode performance and allow for its application in different matrices. Development of sol-gel electrodes is a promising strategy to develop.

This study describes the use of metalloporphyrins [Mn(III)TPP-Cl] in the development of a new anion-selective electrode for valproate determination using two different polymeric supports, *i.e.*, polyvinyl chloride (PVC) and for the first time a ceramic membrane (sol-gel) based on methyltriethoxysilane (MTES). PVC and sol-gel conventionally shaped electrodes presented a slope of -61 and a LPD of $5x10^{-6}$ and 60.3 mV/dec and $1x10^{-4}$ mol l^{-1}, respectively. Different selectivity characteristics of electrodes based on Mn(III)TPP-Cl prepared with PVC or MTES were obtained for several interferents. For the sol-gel electrode, only salicylate interferes.

Both types of membranes were coupled to a Sequential Injection System (SIA) for the direct determination of valproate in pharmaceutical formulations. The association of the new sensing specie Mn(III)TPP-Cl with the sol-gel support inserted in an SIA system provided potentiometric sensors with an analytical range of $1x10^{-3}$- $5x10^{-2}$ mol l^{-1}, with a sample rate of 50 samples/h and a sample and carrier consumption of 140 and 3300 µl/determination.

DETERMINATION OF THYROXINE HORMONE WITH A Eu(III) CHELATE: A SPECTROFLUORIMETRIC STUDY

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Lanthanides represent a unique class of luminescent molecules. Europium (III) and Terbium (III) form highly fluorescent chelates with many organic ligands. The chelates show large stokes shifts, narrow emission bands and long fluorescence lifetimes. Europium aquo ion forms stable mono, bis and tris complexes with pyridine-2,6-dicarboxylic acid (PDA). Thyroxine (Thy) (3,5,3',5'- tetraiodothyronine) is the principle hormone of the thyroid gland. Thy has L and D forms. The L form is twice as active physiologically as the racemic product. The D form has very little activity.

A method is described for the determination of Thy using its quenching effect on the Eu(PDA)33- tris complex fluorescence. The relative fluorescence intensities are measured at fixed $\lambda_{exc} = 282$ nm, $\lambda_{em} = 615$ nm by titrating the metal complex with Thy in piperazine buffer solution at pH 6.5. Data point to an associative type of reaction of two molecules valid between $0.0 < R < 1.0$, R being the mole ratio of Eu(PDA)33 to Thy. Over this ratio and up to $R >> 1.0$, collisional quenching of the Eu(PDA)33 complex ion emission is seen as a result of heavy atom effect, intermolecular energy transfer playing the main role. This is also confirmed by Stern-Volmer Equation. In optimised experimental conditions, the L form of Thy is quantified in the range 2.0×10^{-5} - 7.1×10^{-4} *M* with a relative error of ±1.17%.

ELECTROCHEMICAL AND SPECTROSCOPIC STUDIES OF THE INTERACTION OF PROFLAVINE WITH DNA

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The interaction of small electroactive molecules with DNA is of interest for therapeutic and scientific reasons. Nucleic acids interact reversibly with a broad range of chemical species. These interactions may also be used for conformational recognition to find new structures of DNA and sequence-specific differences along the helix of a DNA molecule. In addition to potential therapeutic applications, many small molecules capable of recognising DNA due to their electroactivity are anticipated to be useful in analytical applications.

In this study, the interaction of proflavine with DNA has been investigated using voltammetric and spectroscopic methods as well as viscosity measurements. Shifts in peak potentials in cyclic voltammetry, spectral changes in UV absorption titration, increase in viscosity of DNA and the effect of ionic strength on the binding constant strongly support the intercalation of proflavine into the DNA double helix. Furthermore, the binding constant and binding site size for the interaction of proflavine with DNA were obtained.

Poster session 3

Environmental, pharmaceutical and clinical analysis

Chairperson
I. Terenetskaya

AN INTEGRATED APPROACH FOR ENVIRONMENTAL PLANNING AND RESTORATION

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To correctly set up action plans and policy implementation for land and environment, there is an outstanding need for an integrated approach. This ought to reinforce a centralised information and to establish an effective channel of communication among information systems for environment, society and government entities. Environmental issues should not be considered only a concern to deal with in the framework of land planning; it implies an ecological re-foundation of the discipline and adequate information on organisational structures of the natural world (natural cycles, reproductive capacity, carrying capacity *etc.*), as well as of the social and economic ones.

All actions on the environment must respect the ecosystem properties to prevent destructive effects, whose restoration (when feasible) would require larger economic investments. Environmental issues and the limits that they may pose to development should be unconditional inputs for land planning, *e.g.*, to define soil conditions before any choice for human activities (interference, use, settlement) can be done. The environmental understanding must be the essential premise for every planning and land management process in the light of sustainable development. Nevertheless, conceptual and technical tools used to understand natural phenomena are often inadequate. When existing, the scientific community of land science experts, who should plan the complex co-evolutionary process unceasingly linking social and environmental systems, scarcely acquaints them. In this context, monitoring and control activities should not only verify whether prescriptions are achieved; they should also facilitate the evolution from the control/prescription approach to another one, more focused on the control/information concept. They should be placed within a wider correlation system, *e.g.*, the DPSIR framework, as described by the European Environment Agency (EEA), *i.e.*, the feedback mechanism based on a chain of causal links starting from the identification of Driving forces, Pressures and changes in the State of the environment, leading to the definition of Impacts on ecosystems and society and, finally, prompting political Responses.

According to this multidisciplinary approach, which also represents a conceptual background of the Italian and European laws for water protection, the real goal of monitoring should be the increase of the environmental information to understand the state and evolution of ecosystems by means of the acquisition of complex sets of selected indicators. The availability and dissemination of information get a crucial importance through an awareness system capable of representing land and environmental reality with its complexity and according to different points of view. The information system should not, therefore, simply depict environmental reality, but also, contemporarily, describe government entity, with its diversity of interpretations coming from the different expertise, exerted functions and adopted tools. The study and the application of this approach at local and/or regional scale has been performed by a working group on some Italian river basins, as it is the case of the Tiber River. Main results and management perspectives in this context are presented.

BIOMONITORING OF WATER QUALITY OF ALBANO LAKE (CENTRAL ITALY)

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The water quality of the Albano lake (central Italy) was evaluated by means of some biological parameters considered to be indicators of organic pollution and, more in general, of environmental stress. The above lake, in the vicinity of Rome, was chosen as a test site since it is a celebrated place for tourism.

Phytoplankton characteristics and related photosynthetic pigments, zooplankton characteristics and microbial contamination, were investigated by biological techniques., Water samples were monthly collected from June 1999 to April 2000 at the same station and depth of the lake for phytoplankton and zooplankton analysis. In addition, water samples were collected every six months for microbial analysis. In the latter case, sampling was carried out at three different depths and also sediment samples were collected to determine *Clostridium* spore presence.

Significant differences in the biological parameters were found between samplings conducted in different months and, only as regards microbial contamination, at different depths. These findings point to water organic pollution especially during summer, probably caused by tourism activities. The large set of biological parameters used allowed for a thorough water quality assessment.

COMPARING BIOTIC INDICES USED TO EVALUATE WATER QUALITY OF FOSSO CASACCIA (ITALY) AND RIVER WELLAND (UK)

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A practical comparison was made between biotic indices, based on freshwater macroinvertebrate communities, as developed in different European countries. The English Trent Biotic Index (TBI) and the Lincoln Quality Index (LQI), the Italian Extended Biotic Index (IBE) and the Iberian Biological Monitoring Working Party (BMWP) score were applied to data collected from rivers in Italy and England (Fosso Casaccia and River Welland, respectively).

Analysis using the TBI appeared to be unfit to detect changes in quality trends at sites impacted by sewage effluents in either river. All the other indices, however, were fit for purpose when applied to the River Welland, with a high degree of similarity in terms of intercalibrated quality classes. In the Fosso Casaccia, the index responses were more diversified, but this was probably due to excessive disruption of the physical habitat, non-point contaminants, general paucity of flow and overwhelming changes in the biota due to point-source impacts. End-point quality classes were, however, comparable.

It is clear that further studies to evaluate the applicability and comparability of indices outside the country of origin, over a variety of environmental settings, would be quite useful. Intercalibration might therefore become a practical possibility.

ASSESSMENT OF POTENTIAL RIVER POLLUTION FROM NON-POINT SOURCES

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Assessment of water bodies' pollution from non-point sources is a complex, data and time-consuming task. The Potential Non-Point Pollution Index, PNPI, is a tool designed to assess the global pressure exerted on rivers and other surface water bodies by different land uses. The main feature of PNPI is the low data request that tries to overcome the problem of scarce monitoring data that still affects many countries in the world. The low data request implies a simplification of the physical reality that authors counterbalanced using an "expert system" approach. The system bypasses the accurate representation of the physical reality to assess globally the pollution potential of different areas according to scientists' judgement.

The scientific community proposes many models for the control of diffuse pollution dynamics. Most of them can be grouped into two broad categories: statistical models and physically based models. PNPI belongs to neither of the above-mentioned groups.

PNPI is a GIS-based, watershed-scale tool designed to inform decision makers and public opinion about the potential environmental impact of different land management scenarios. PNPI innovative approach applies the multicriteria technique to pollutants dynamics and water bodies' health. Pressure of diffuse pollution on water bodies coming from land units is expressed as a function of three indicators: land use, run-off and distance from the river network. They are calculated from land use data, geological maps and a Digital Elevation Model (DEM). The weights given to different land uses and to the three indicators were set according to experts' evaluations and allow the value of the PNPI on each node of a grid representing the watershed: the higher the PNPI of the cell the heavier the potential impact on the river network to be calculated. The output of the calculation is presented in the form of maps that highlight areas that are more likely to produce pollution. Another output shows river stretches that undergo major pressure from diffuse sources of pollution.

In the authors' view, the explicit link between land use and potential pollution on which PNPI is based, along with its high communication potential, makes it particularly interesting for a participatory and integrated approach to land management and water bodies environmental protection.

IMPROVED PURGE-AND-TRAP INJECTION GAS CHROMATOGRAPHY MASS SPECTROMETRY ANALYSIS OF VOLATILE HALOGENATED HYDROCARBONS IN WATER

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Among priority environmental issues, there is a growing concern about the fate of anthropogenic compounds such as Volatile Halogenated Hydrocarbons (VHHs). These substances include Volatile Chlorinated Hydrocarbons (VCHCs) and Trihalomethanes (THMs), which are typical contaminants of water. VCHCs (*e.g.*, chloroform, 1,1,1-trichloroethane, tetrachloromethane, 1,1,2-trichloroethylene and tetrachloroethylene) are used in a wide variety of industrial and commercial processes. These compounds are considered as global contaminants and pollution indicators in remote areas. Their volatility contributes to global diffusion because, while non-volatile compounds are deposited and accumulate close to their source, more volatile compounds undergo long-range atmospheric transport before deposition in colder regions. THMs (*e.g.*, chloroform, bromodichloromethane, dibromochloromethane and bromoform) are a class of disinfection by-products formed when chlorine reacts with natural organic matter and bromide found in drinking water. Chlorination is only allowed in tap water and thus THMs only occur in that kind of water. The most common technique for analysing VCHCs and THMs in water (and the one expressly provided for by the law) is a liquid-liquid extraction with an organic solvent (hexane or pentane) and a subsequent analysis of the extract through Gas Chromatography with an Electron Capture Detector (ECD-LLE-GC). Unfortunately, the ECD-LLE-GC technique does not provide unequivocal identification of substances nor does it accurately quantify some compounds. Of the other techniques provided for by the law, Purge-and-Trap Injection Gas Chromatography Mass Spectrometry (PTI-GC-MS) is the most satisfactory in terms of qualitative and quantitative analysis and sensitivity level. However, even if current PTI-GC-MS methods can be used to determine a wide range of purgeable organic compounds, they are not specific for VCHCs and THMs. Determining VCHCs and THMs entails relatively high Limits of Detection (LoDs) that range from 0.1 to 0.04 μg/l. Although these LODs are adequate in terms of the law, they do not allow for the analysis of water that has only been contaminated at low levels (*e.g.*, samples from remote areas).

This work proposes a technique based on modified PTI-GC-MS in the SIM mode, specific for the analysis of VCHCs and THMs in water. The modification involves sample introduction into the purge-and-trap injector in order to avoid any air intake in the system that could alter the final results. Moreover, operating on cold trap temperature, even sparkling mineral waters can be analysed, thus avoiding CO_2 interferences during the cryo-concentration phase. Modified PTI-GC-MS overcomes the limitations of ECD-LLE-GC and lowers the conventional LoDs of PTI-GC-MS. This method is more accurate and selective than previous procedures for all examined compounds and allows for correct on-line analysis even for chloroform and 1,1,1-trichloroethane, that are not detectable with ECD-LLE-GC. This method has been successfully used for real samples such as tap water, mineral water and Antarctic snow.

CHARACTERISATION OF ENVIRONMENTAL COLLOIDS BY ON-LINE SEDIMENTATION FIELD FLOW FRACTIONATION-ATOMIC ABSORPTION SPECTROMETRY

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Colloidal particles (size range 0.001-1.0 μm) play an important role in many environmental processes: they can strongly bind pollutants and thereby facilitate their transport (*e.g.*, in river systems or in the atmosphere) or enhance their retention (*e.g.*, in soils), depending on the degree of mobility of the colloids. Many methods have been developed to characterise colloidal matter, but the sizing of materials in the colloidal range and their chemical characterisation is difficult with just one method alone. Using Field-Flow Fractionation (FFF) methods, several size fractions can be collected (across the sample size distribution) for subsequent observations, such as heavy metal load determination. However, the small amount of sample that can be processed in a single separation run is a limiting factor. Elemental characterisation of the Sedimentation Field-Flow Fractionation (SdFFF) separated particle fractions thus requires a very sensitive analytical method in order to avoid time-consuming concentration steps and the ensuing further sample handling procedure.

In the present work Electrothermal Atomisation Atomic Absorption Spectrometry (ETA-AAS) equipped with a Capillary Injection Device (CID) is presented as an on-line detection system for SdFFF. The CID allows for the direct introduction of up to 30 ml of the analytical solution into the graphite furnace, thus preconcentrating the colloidal particles on the furnace walls. The determination of elements such as Cd, Co or Fe even at the pg/g level in the eluate of SdFFF containing separated fractions of environmental colloids has been proved to be possible, thus avoiding off-line manipulations.

TOTAL AND PARTIAL DIGESTION OF SEDIMENTS FOR EVALUATION OF TRACE ELEMENT ENVIRONMENTAL POLLUTION

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It is well-known that the correct selection of the procedure for the digestion of sediments is a key step for the environmental interpretation of the results in the assessment of pollution by trace elements. At present, however, there is no consensus at the international level for the definition of a unique digestion method. The lack of a harmonized digestion method does not allow for a consistent interpretation and a reliable comparison of results reported in different parts of the world.

In this work different procedures were applied and compared for the preparation of sediment samples. The samples were taken in the Bay of Cienfuegos, in the southern central part of Cuba, which is a semi-closed bay connected to the Caribbean Sea by a 3 km long narrow channel. The bay is subject to anthropic impact, primarily because of the sewage outlet of Cienfuegos city and of the industrial area. The samples considered are representatives of different pollution degree (low, medium and high) as ascertained through previous investigations in this bay.

In particular, four procedures were taken into consideration and compared in this study: a total digestion with HNO_3-H_2O_2-HF with the aid of microwave irradiation and three different standardised methods of fractionation, namely the three-step sequential extraction (BCR), the EPA 3050B and the ISO/DIS 11466.2. The determinations were carried out by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after total digestion and application of sequential extraction procedure (BCR), whereas Flame Atomic Absorption Spectrometry (FAAS) was used after application of the EPA 3050B and ISO/DIS 11466.2 procedures. Copper, Ni and Pb were taken in consideration as representative of anthropic impact in the Cienfuegos bay. Laboratory performance was evaluated by recovery assay and analysis of appropriate Certified Reference Materials (CRMs), where available.

The results of the four procedures considered allow the general anthropogenic impact on the studied area to be asssessed; even if differences in the efficiency of extraction of trace elements were noticed for the various procedures, however there was consistency among different fractionation procedures in the identification of the pollution degree.

INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY DETERMINATION OF EXTREME U ISOTOPE RATIOS USING A NEW MICROFLOW NEBULISER SYSTEM

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Double-focusing Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was coupled with the new microflow nebuliser system APEX for U isotope analysis, in particular for the determination of extreme $^{236}U/^{238}U$ isotope ratios. The new sample introduction system allows the sensitivity to be improved by a factor of six and oxide and hydride molecular ions to be reduced by up two orders of magnitude. Thus, a sensitivity of more than 5000 counts/fg can be achieved for U, which is significantly higher than the sensitivity provided by other nebulisers. The abundance ratio sensitivity for $^{236}U/^{238}U$ is as low as $3x10^{-7}$ at mass resolution m/Δm = 4000. ICP-MS coupled with an APEX system therefore allows the measured concentration range of ^{236}U to be increased by about one order of magnitude.

The isotope ^{236}U was used to monitor spent U (*i.e.*, the main nuclear fuel component having been burned in a nuclear reactor) in soil samples collected in the vicinity of the Chernobyl Nuclear Power Plant (NPP) from nuclear fallout using ICP-MS. A comparison of the $^{235}U/^{238}U$ isotope ratio measurements with the $^{236}U/^{238}U$ ratio provides more sensitive and accurate determinations of the portion of spent U in soil samples down to 0.01% by using the last mentioned isotope ratio . Concentrations of ^{236}U in the upper soil layers (0-10 cm) of samples from Belarus were found to be 10-13 g/g to $8.1x10^{-10}$ g/g depending on the distance to the Chernobyl NPP.

ICP-MS coupled with APEX can also be used as a very powerful method for ultratrace isotopic analysis of long-lived Pu nuclides (after chemical separation of Pu from U with a decontamination factor of ~10^6 to 10^7) in environmental monitoring forensic studies.

ARTIFACTS AFFECTING ATMOSPHERIC PAHS SAMPLING AND A POSSIBLE WAY TO PREVENT THEM

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Atmospheric Polyaromatic Hydrocarbons (PAHs) (both in the gas and particle phases) were collected at Montelibretti (a rural area 30 km NE of Rome) by coupling the annular denuder technique with air filtration through inert membrane filters. The organic burden was recovered from the collecting media through solvent extraction. PAHs were purified from possible interferents by means of column chromatography and finally determined by Gas Chromatography-Mass Spectrometry (GC-MS) operated in the Electron Impact, Selected Ion Monitoring (EI-SIM) mode. For quantification, perdeuterated congeners with a wide spectrum of volatilities were spiked into the extracting solvent. The entire procedure developed allowed possible biases occurring at the sampling step to be highlighted, since both positive and negative artifacts develop and heavily affect both qualitative and quantitative determinations.

The concern of researchers is usually focused onto PAHs with molecular weights exceeding 226, which are regarded as associated to particulate matter. Thus, the PAHs content in air is often investigated by collecting soot, whilst only on few occasions the semi-volatile fraction is collected by using a backflow cartridge (polyurethane foam or organic resin). This approach appears reliable for very high-boiling species, which remain bound up in aerosol particles, if they do not undergo appreciable decomposition by atmospheric oxidants; however, it is not valid in general and, at best, provides the total (gas plus particulate) presence of PAHs in air. This is the reason why a new procedure has been conceived, by introducing high-efficiency annular denuders upstream of the particle filter to collect selectively gas-phase species. For this purpose, the best features seem to be exploited by XAD-4, a porous macroreticular polystyrene-divinylbenzene resin.

In this study, a pair of XAD-4 coated denuders was used in front of inert quartz membrane filters and air was aspirated at 5 l min-1 through this sampling system. Two sampling lines were used in each test, both equipped with a front KI denuder to cut-off ozone and oxidants, thus preventing partial decomposition of reactive PAHs. In each line two filters were accomodated into a dual filter holder, the back one coated with XAD-4 to capture the fraction of native particulate PAHs desorbed during sampling.

This investigation confirmed that XAD-4 is a good adsorbent for vapour-phase PAHs, although it is not a chemical sink for organics. While good trapping efficiencies for gaseous PAHs ranging from methylnaphthalenes to benzo(a)pyrene were found with air volumes sampled below 18 m^3, the use of the back filter revealed significant losses of three- and four-ring PAHs from the front filter. Only when the sample volume was reduced to 2 m^3 and, consequently, sampling duration was not exceeding 6 h, it was possible to efficiently collect naphthalene on the front denuder and particle-associated semi-volatile PAHs onto the front filter.

PAHs concentrations in air resulting from the use of this collection train were consistent with previous studies, carried out on volatile and high-boiling PAHs collected on diffusion samplers or filter systems, respectively.

ASSESSMENT OF PARTICULATE MATTER EMISSIONS FROM TURBOGAS PLANTS

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The production of very high amounts of energy required by developed countries implies necessarily the use of power plants more and more efficiently and respectfully of the environment. In such framework the environmental impact assessment has gained a very important role. Indeed, the thermoelectrical plants gives place to a combustion process which produces and consequently emits in the atmosphere gaseous or particle compounds able to negatively affect the surrounding environment, thus endangering public health. Recently, the Italian Government has planned the increase of the energy fraction coming from combined cycle turbogas plants. Such action allows for a higher fuel's differentiation, for a very high electrical efficiency (greater than 55%) and for a very low expected environmental impact.

The Directive 2001/80/EC of the European Parliament of 23 October 2001 on the "limitation of emissions of certain pollutants into the air from large combustion plants" indirectly, but clearly, states the lack of particulate matter emissions from such plants. The Directive does not set any limit for the particulate matter emitted by the gas turbine (Part B, Attachment VII). It appears clear that this decision originates from the assumption that the turbogas plants do not emit particulate matter.

For all the above mentioned reasons the collection of further data concerning atmospheric pollutants' emissions and micropollutants' emissions coming from the Natural Gas (NG) thermoelectrical plants turns out to be necessary in order to add quantitative elements to the discussion regarding the need of building new combined cycle plants.

The Institute for Atmospheric Pollution of the Italian National Research Council carried out numerous samplings of the emissions coming from the ENEL combined cycle plant in Porto Corsini (Ravenna). The samplings involved a 400 MW power generator in order to assess the extent and the type of emissions of particulate matter and other macropollutants (CO, CO_2, NO_x, SO_2) and also of micropollutants normally monitored on this kind of plants, such as Volatile Organic Compounds (VOCs), Mg and aldehydes.

This study proves that the use on a large scale of the combined cycle plants allows for an appropriate diversification of fuels and ensures a very high efficiency and a moderate environmental impact. The amount of particulate matter emitted by a turbogas plant is negligible and in accordance with the European Community statement (no limits for the particulate matter emissions from such plants).

ORGANIC FRACTION COMPOSITION OF ATMOSPHERIC AEROSOL AT THE AIR POLLUTION REFERENCE SITE IN ROME, ITALY

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The organic fraction of particulates (both total suspended matter and the PM10 fraction) was determined in Rome at the Reference Site of the city network for atmospheric pollution. The campaign was conducted for an entire year (from April 2003 to April 2004), by collecting soot through two sequentially sampling sets, operating at medium flow (1.0 m^3 h^{-1}). While the former enriched total suspended particulates, the latter was equipped with an inertial impactor capable to collect selectively the particles with aerodynamic diameter not exceeding 10 µm.

After collection (each starting at 00:00 h), particulates were pooled into four groups per month and processed for chemical analysis. For this purpose, after spiking loaded filters with an internal standard mixture containing perdeuterated congeners of analytes, the organic burden was solvent extracted (by soxhlet), fractionated by elution through a neutral alumina column (*iso*-octane and dichloromethane/*iso*-octane in sequence) and analysed by means of Gas Chromatography Mass Spectrometry (GC-MS) operated in SCAN (*n*-alkanes) or SIM (PAHs, nitro-PAHs) mode.

By looking to the results obtained, one can observe that: *i*) benzo(*ghi*)perylene was in all cases the most abundant PAHs congener investigated; *ii*) benzo(*a*)pyrene, at monthly averages, was always at concentrations lesser than the old reference value reported by the Italian legislation (1 ng m^{-3}), but sometimes exceeding the value valid in the near future (0.5 ng m^{-3}); *iii*) most of PAHs burden was accumulated in the fine PM10 fraction; *iv*) based upon the ratios between pairs of PAHs used as diagnostics to highlight the occurrence of hard photochemical processes, the particulates reaching the test site were freshly emitted; *v*) surprisingly, the semivolatile congeners appeared relatively more abundant in the warm months, suggesting the influence of a season-modulated source; *vi*) based upon the PAHs fingerprints observed and literature data, the principal source for PAHs seemed to coincide with vehicles, diesel-engined in particular; *vii*) *n*-alkanes show a bimodal distribution, with the two maxima coinciding with C20 and C27 homologues. The light portion of *n*-alkane set was consistent with a petrogenic origin, whilst in the range higher than C25 the influence of natural sources could be associated to predominance of odd-carbon numbered homologues; *viii*) as regards nitro-PAHs, the concern was focused primarily on nitrofluoranthene and nitropyrene isomers, due to their strong mutagenic potency. Aerosols resulted usually richer of 2-nitrofluoranthene than of 1-nitropyrene, assigning to oxidation processes a role more important than that of direct emission regarding the presence of nitroarenes in the air; *ix*) although present at trace levels (<< 1 ng m^{-3}), however nitro-PAHs were often at levels similar to benzo(*a*)pyrene; thus, their contribution to long-term ambient toxicity could not be neglected.

A CHROMATOGRAPHIC STUDY ON RED WOOD DYES

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During the Middle Ages many natural red and purple dyes such as indigo, Tyrian purple, madder and carmine, lichen dyes such as orchil and tree dyes such as brasilwood and logwood were employed for a variety of purposes. Primarily, sappanwood (extracted from *Caesalpina sappan L.*) was imported into Europe from India, Malaysia and Sri Lanka: the dye named brazil by the Portuguese gave its name to Terra de Brasil where many dyewood trees (*Caesalpinia echinata L.*) very similar to brazil used to grow. Like sappanwood, it became quite an expensive coloring material for cotton, wool, leather, silk and inks, even though other animal and vegetable extracts were considered to be superior permanent dyes.

Many other leguminous red-purple dyes were introduced into Europe at that time, such as logwood (*Haematoxylon campechianum L.),* brazilwood (*Caesalpina brasiliensis L.),* pernambucowood (*Caesalpina crista L.* and brasiletto *(Haematoxylon brasiletto L.).* All of them are characterised by a different quantitative composition of neoflavanoid and isoflavonoid compounds. The main neoflavonoid components hematoxylin (3,4',5',7,8-pentahydroxy-2',3-methylen-neoflavan) and brazilin (3,4',5',7-tetrahydroxy-2',3-methylen-neoflavan) undergo a partial oxidation to hematein and brazilein due to natural ageing or simply due to exposure to the air, giving a characteristic purplish-red color.

A reliable identification of historical dyes entails the chemical characterisation of dye sources and an understanding of the chemical changes caused by ageing. This study deals with the characterisation of flavanoid organic dyes by using High Pressure Liquid Chromatography (HPLC) and Gas Chromatography (GC) coupled with Mass Spectrometry (MS). The main aim is to assess stable molecular markers which may help to identify leguminous red-purple dyes in ancient samples. The most significant results obtained for reference and old samples provided by the Opificio delle Pietre Dure are presented.

AN INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY STUDY OF THE ABUNDANCE OF As, Hg, Pb AND Sb IN A CONTAMINATED AREA OF CENTRAL ITALY

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The purpose of this study was to quantify the level of contamination and Hg pollution in a mining area of Tuscany, Monte Amiata (Italy). Contamination of this area occurred during the mining and processing of cinnabar (HgS) over a prolonged time. It resulted that, in this zone, Hg exceeds the tolerance limit. It has been also ascertained that even if the industrial processing of cinnabar in this zone has been abandoned since more than ten years, local pollution is still substantial.

A laboratory method was developed for As, Hg, Pb and Sb monitoring also at very low concentrations. The method is based on Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The method has been applied also to the determination of chemical species of Hg in water samples from the same area.

AN INVESTIGATION ON THE ORGANIC RESIDUES IN ARCHAEOLOGICAL GLASS VESSELS FROM POMPEII AND OPLONTIS (ITALY)

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The organic residues associated with archaeological objects may contain a wide variety of natural substances. The chemical analysis of these substances provides archaeologists with very useful data on diet, technology and everyday life in past societies. The characterisation of organic residues is particularly complex due not only to the chemical complexity of the original materials, but also to the fact that the materials underwent transformation and processing before use and then aged and degraded while buried.

In the framework of an Italian survey on the characterisation of glass vessels of the Roman age recovered at Pompeii and Oplontis (Italy), the chemical composition of organic residues was investigated by Gas Chromatography Mass Spectrometry and IR spectroscopy. Most of them were identified as mixtures of vegetable oils with Pinaceae diterpenic resin and beeswax, while one was a single material, identified as palm oil.

DETERMINATION OF OZONE IN A MEDITERRANEAN FOREST CANOPY AT DIFFERENT LEVELS USING DIFFUSIVE AND CONTINUOUS SAMPLING

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The formation of O_3 is a large-scale phenomenon and this secondary pollutant has generally a quite uniform spatial distribution. However, its concentration is likely to differ with location in a forest and for different species, because vegetation has different properties and can – as a whole – behave as a sink or not. Decay of forests in relation to O_3 has mainly been studied in forest clearings and there are only a few studies on the vertical distribution of O_3 in forests. This study reports on a campaign for O_3 in a forest characterised by the prevailing presence of holm-oak trees. Two methods for measuring O_3 were used, namely, long-term monitoring using diffusive samplers at three heights within the canopy and continuous monitoring at two heights using the UV method. Average results over one week of O_3 above and below the canopy from the diffusive samplers were compared to those obtained using the automatic analyser at the same levels. A good correlation between the two sampling techniques was found. Continuous monitoring showed a daily cycle with a midday maximum and a nocturnal minimum. While the forest floor consistently had the lowest O_3 concentration, there were no differences during most day-time hours. The midday maximum is clearly due to downward mixing of O_3 rich air from above. The night-time O_3 decay within the canopy is the result of dry deposition of O_3 and most likely due to reaction with biogenically produced NO. AOT40 within and above the canopy largely exceeded the critical levels. Based on the results obtained, it could be also concluded that diffusive sampling provides a simple and cost-effective way of monitoring gradients of O_3 in a forest. Diffusive samplers can be implemented at a fraction of the cost of continuous monitoring and can be used where continuous measurements are unfeasible and can cover a virtually undefined number of different sites. Diffusive samplers can be used at multiple locations within a certain area to identify locations of high exposure to O_3 and elucidate the spatial and temporal gradient of O_3 concentration. This is particularly beneficial in areas with a complex terrain as it is often the case in Italy. Diffusive samplers could be used in a variety of further studies to help identify the scale of ozone pollution in forests.

MONITORING OF HYDROGEN SULPHIDE BY DIFFUSIVE SAMPLING

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Hydrogen sulphide is not routinely measured in urban air, even if recent findings have shown that its importance is growing due to emission from catalytic converters. A simple, inexpensive diffusive sampler was developed for monitoring H_2S. In principle, diffusive sampling represents a perfect tool to characterise those areas where the limit values are expected to be exceeded and/or where alternative assessment methods are needed. Compared with active sampling, the major advantages of the method are cost effectiveness, simplicity and suitability for mapping large areas. The sampler is a modification of the open-tube design (Analyst®) obtained by using a filter paper impregnated with a solution based on the use of $AgNO_3$ as a component of a suitable mixture. In the presence of H_2S a stain of Ag_2S darkens the absorbing pad. The amount of H_2S is determined by reflectance measurements using a home-built, low-cost portable reflectometer. The decrease in reflectance is proportional to the concentration of H_2S. The use of a simple reflectance reading method cuts off the time-consuming, expensive laboratory analysis necessary for diffusive samplers developed to collect other pollutants. The diffusive sampler was tested in chamber and field trials. Laboratory and field tests concerning sorption efficiency, capacity and effect of humidity were carried out. The effect of wind turbulence, the interference of NO_2 and the self-consistency of the method were also elucidated. A typical application of the developed diffusive sampler entails a sampling time of at least one month at concentrations usual for a polluted atmosphere (about 500-1000 pg g^{-1}). The results obtained are reported and discussed.

VARIATIONS IN THE LEVEL OF SOME TRACE ELEMENTS IN HUMAN HAIR OF PARTICIPANTS IN THE ITALIAN EXPEDITIONS IN ANTARCTICA

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As a part the Italian National Programme of Research in Antarctica (PNRA), a monitoring study has been undertaken to assess possible variations in the concentrations of selected trace elements in human hair of participants in the Antarctic expeditions. Differences in concentration could be related to the extreme environmental conditions and changes in lifestyle to which participants in the expeditions are subject, as some evidence in previous investigations seems to suggest.

The present study regards samples collected just before and at the end of the 2003-2004 expedition to the Italian-French Base of Dome C. Seven essential elements were taken into account, namely, Ca, Cu, Cr, Fe, Mg, Mn and Mo. Prior to the analysis, the hair samples were washed to remove exogenous substances from the hair surface that may interfere with the determination of the endogenous concentrations of the elements of interest. Samples were digested by microwave-assisted acid treatment. The determination of the content of trace elements was performed by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and Dynamic Reaction Quadrupole Cell Inductively Coupled Plasma Mass Spectrometry (DRC-Q-ICP-MS). Data obtained were statistically treated by using the non-parametric Friedman test. Calcium (675 and 569 $\mu g\ g^{-1}$) was the only element with $P \leq 0.05$ as the significance level.

These results were compared with data obtained from hair samples collected during the 2002-2003 expedition to the Italian Base of Terra Nova Bay (now Mario Zucchelli Base). In that study the elements with significant differences in the concentration values before and after the expedition were Ca, Cu and Mg (Ca, 563 and 422 $\mu g\ g^{-1}$, Cu, 11.5 and 10.7 $\mu g\ g^{-1}$, Mg, 73 and 64 $\mu g\ g^{-1}$).

The observed differences in elemental concentrations, especially as far as Ca is concerned, might be ascribed to different causes regarding not only the diet intake, which generally resulted adequate, but also the psychological and health status of individuals, the environmental impact on human adaptation and the activities of the subject during the expedition. These assumptions need to be corroborated by more abundant data in future campaigns through a higher number of samples.

CONTRIBUTION OF THERMAL ANALYSIS TO DRUGS SAFETY. COMPARISON OF STABILITY DATA OF PENICILLINS AND CEPHALOSPORINS

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It is largely recognized that the availability of a great number of drugs and pharmaceutical formulations certainly contributed to extend the life expectation and at the same time to improve the quality of life. Therefore, the pharmaceutical industry is called to solve many problems related to the development of new active components and formulations as well as to evaluate the stability of commercial pharmaceutical drugs. Thus, the evaluation of stability of active components both pure and contained in pharmaceutical formulations is one of the most current problems of the pharmaceutical industry.

Thermal analysis is a routine method for the analysis of drugs and substances of pharmaceutical interest. Moreover, it is also well known that the chemical reactivity of drugs, either pure or mixed, can lead to undesired and uncontrolled reactions when temperature increases. For this reason it is necessary to undertake thermal stability studies that usually requires several months. The classical stability studies imply long-lasting observations that cannot be completely replaced by thermal analysis. However, this latter technique can provide an early alert and indicate the most favourable pathways to achieve a successful formulation, as kinetic results obtained by thermal analysis allows stability data to be obtained more rapidly.

A thermal analysis investigation on penicillins and cephalosporins was carried out in the past and is currently being investigated in order to better understand their thermal behaviour and stabilities. After dehydration penicillins and cephalosporins undergo exothermic multi-step decomposition processes under a stream of air. The temperature dependence of the decomposition rate is strictly related to the activation energy, which is reliably obtained from dynamic model-free method that uses thermal analysis data performed at different fixed heating rates, β.

Due to the complexity of the decomposition patterns the kinetic method proposed in this research is mainly based on the Kissinger's equation. Onset decomposition temperatures and activation energies were used to predict the thermal stability of the investigated compounds and the results obtained are compared and critically discussed.

A HIGHLY SENSITIVE CHEMILUMINESCENCE ASSAY FOR THE EVALUATION OF PHARMACOKINETICS OF RASBURICASE, A RECOMBINANT URATE OXIDASE

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Hyperuricemia might have a pathogenetic role in hypertension and progression of Chronic Kidney Disease (CKD) and could be a marker of cardiovascular risk. Allopurinol, an inhibitor of xanthine oxidase (the enzyme responsible for the conversion of hypoxanthine to xanthine and of xanthine to uric acid), is the drug of choice for the chronic treatment of hyperuricemia, but its use is limited by 2-4% side effects. Recently, rasburicase, a recombinant urate oxidase, has been developed and licensed for the treatment of acute hyperuricemia in patients with hematological malignancies. Urate oxidase is an enzyme lacking in humans, but present normally in other mammals, which is responsible for rapidly converting the poorly soluble uric acid to highly soluble allantoin, easily excreted by the kidneys. Administration of rasburicase causes a rapid and pronounced reduction in plasma uric acid concentrations. This enzyme may thus represent a valuable alternative for the control of uric acid levels in CKD patients with allopurinol intolerance.

In order to define chronic treatment protocols with rasburicase, its pharmacokinetics in hyperuricemia CKD patients has been investigated. Until now, the pharmacokinetic profiles of rasburicase were determined by using immunometric techniques. However, antibodies against urate oxidase are not commercially available. Moreover, chronic treatment protocols require the continuous monitoring of the enzyme levels. A rapid, inexpensive and sensitive microtiter plate-based chemiluminescent assay for the quantification of rasburicase levels in serum has been thus developed. The assay relies on the chemiluminescent reaction of H_2O_2 produced by the enzyme-catalysed oxidation of uric acid with bis(2,4,6-trichlorophenyl)oxalate (TCPO) in the presence of the energy acceptor dipyridamole. Analysis is directly performed on diluted (1:100-1:200 *v/v*) serum samples in order to decrease the matrix effect due to sample components. Nevertheless, the high detectability of the chemiluminescent signal allows for the determination of urate oxidase with a Limit of Detection (LoD) of about 50 ng/ml^{-1} (during treatment, the peak serum levels of rasburicase are in the range of 1500-3000 ng/ml). Since the chemiluminescence assay measures the activity of the enzyme rather than its concentration, it also gives direct information about the therapeutic effect of the enzyme. In addition, the assay could be easily automated using commercially available sample handling systems.

The pharmacokinetics profiles of rasburicase in hyperuricemia CKD patients, evaluated after a 0.2 mg/kg body weight single-dose administration, showed a half-life of about 15 h, in good agreement with the data previously reported in the literature. After rasburicase administration, uric acid concentrations decreased below the LoD of the conventional clinical chemistry assays for at least three days. Therefore, rasburicase seems to be a highly effective drug in hyperuricemia patients with CKD and, once suitable treatment protocols are defined, it could be used in those cases when allopurinol intolerance occurs.

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