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Number of articles in scientific journals: **9** (2008) and **8** (2009)

Aim

Analytical methods, using liquid chromatography with diode array, mass or fluorescence detectors or gas chromatography coupled with electron capture detector or mass detector, for the determination of pesticides, polycyclic aromatics hydrocarbons, toxins or antibiotics in food samples (vegetables, bread, fish,…), atmospheric particulate samples and water and wastewaters were developed. Significant parameters affecting extraction efficiency were optimized trying different pré-preparation methodologies such as microwave-assisted extraction, solid phase extraction, solid-phase microextraction and QuEChERS (quick, easy, cheap, effective, rugged, and safe).

To reduce sample preparation and time-consuming extractions steps electroanalytical procedures were considered. They also decrease significantly amounts of solvent and require cheaper instrumentation. The electrochemical behaviour and mechanism of some pesticides at a glassy carbon electrode (GCE) and a hanging mercury drop electrode (HMDE) were used with regard to cyclic voltammetry (CV) and square wave voltammetry (SWV) experiments. The methods were applied to waters control. Some of these methods were developed in flow conditions with an amperometric detection to permit determinations at the $\mu g L^{-1}$ level and offer advantages of simplicity, accuracy, precision, and applicability to coloured and turbid samples, and automation feasibility.

The same voltammetric techniques were also applied to pharmaceuticals control and validation of these methods revealed good performance characteristics confirming applicability for the quantification in several pharmaceutical products.

An Approach to Investigate the Importance of Polycyclic Aromatic Hydrocarbons Compounds in Ambient Air

Traffic emissions and tobacco smoke are considered two main sources of polycyclic aromatic hydrocarbons (PAHs) in indoor and outdoor air. In this study, their impact on the level of fine particulate matter (PM2.5) and on the distribution of 15 PAHs on PM2.5 were evaluated and

compared. PM2.5 can penetrate in the deeper parts of the lungs, causing pulmonary diseases, lung cancer and premature mortality.

Outdoor and indoor PM2.5 samples were collected during winter 2008 in Oporto city in Portugal, for sampling periods of 12 hours (indoor) and 24 hours (outdoor). A methodology based on microwave-assisted extraction and liquid chromatography with fluorescence detection was applied for the efficient PAHs determination.

Indoor PM2.5 concentrations were significantly influenced by the presence of traffic and tobacco smoking emissions. The indoor-to-outdoor (I/O) ratio for indoor non smoking PM2.5 concentration indicated the absence of significant indoor sources related with domestic activities.

PAHs with higher molecular weight (5-6 rings), that are classified as carcinogenic, were preferentially found in the outdoor traffic and indoor non smoking sites. The I/O PAHs ratios without the influence of tobacco smoking allowed concluding that PAHs concentrations in indoor air were dominated by outdoor traffic emissions.

The presence of tobacco smoke emissions increased significantly PAHs concentrations on average about 1000 times more, with a significant increase on the concentration levels of PAHs with 3 and 4 rings, when compared with the outdoor profile samples, affected by high traffic intensity. This analysis suggests that tobacco smoking may be the most important source of indoor PAHs pollution.

Goal for 2009:

Characterization of atmospheric deterioration of stone monuments exposed to urban environments

Static and hydrodynamic monitoring of active species based on its electrochemical oxidation/reduction behaviour

The active pharmaceutical ingredients of commercial products can be analysed by electroanalytical techniques, such as voltammetry and amperometry, because of the presence of functional groups in their structure which can be readily oxidised and/or reduced in aqueous media. The studied active pharmaceutical ingredients were the antidepressant

citalopram and the antihypercholesterolemic agent fluvastatin. Using cyclic voltammetry to study the nature of the electrochemical oxidation/reduction process and square-wave voltammetry for quantification purposes, methods were developed for the analysis of these ingredients in commercial formulations. Both glassy-carbon and mercury drop electrodes were applied in these studies. For the quantification of fluvastatin an electrochemical pre-concentration step preceded the square-wave voltammetric scan to further decrease the limit of detection. Amperometric detection, based on the oxidation of the referred substances, was linked to a flow-injection analysis manifold to increase the sample-rate.

Goal for 2009:

Fabrication of new sensors using different chemically modified electrodes (immunosensors, DNA sensors, Self-Assembled Monolayers (SAMs), carbon nanotubes, …) to use in analytical control.

Development of sensitive electroanalytical methods.

Exploration of several electrode construction techniques.

Application of the developed sensors to pharmaceutical, food, medicinal, and environmental samples.

Analysis of polycyclic aromatic hydrocarbons in fish: Evaluation of a QuEChERS extraction method

Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic compounds that are included in the European Union and US Environmental Protection Agency (US EPA) priority pollutant list due to their mutagenic and carcinogenic properties. Excluding smokers and occupationally exposed populations, most individuals are exposed to PAHs predominantly from dietary sources. In the marine environment, PAHs are bioavailable to marine species via the food chain, as waterborne compounds and from contaminated sediments. As lipophilic compounds they can easily cross lipid membranes and have the potential to bioaccumulate in aquatic organisms. Although for most people, fish and seafood represents only a small part of the total diet, the contribution of this food group to the daily intake of PAHs in some individuals may be comparatively important.

QuEChERS method was evaluated for extraction of 16 polycyclic aromatic hydrocarbons (PAHs) from fish samples. For a selective measurement of the compounds, extracts were analysed by LC with fluorescence detection. The overall analytical procedure was validated by systematic recovery experiments at three levels and by using the standard reference material SRM 2977 (Mussel tissue). The targeted contaminants, except naphthalene and acenaphthene, were successfully extracted from SRM 2977 with recoveries ranging from 63.5–110.0% with variation coefficients not exceeding 8%.

The optimized methodology was applied to assess the safety concerning PAHs of horse mackerel (Trachurus trachurus), chub mackerel (Scomber japonicus), sardine (Sardina pilchardus) and farmed seabass (Dicentrarchus labrax). Althought benzo(a)pyrene, which is the marker used for evaluating the carcinogenic risk of PAHs in food, was not detected in the analysed. Significant differences were found between the mean PAHs concentrations of the four groups.

Goal for 2009:

Evaluation of the presence of PAHs in fish muscle, using analytical procedures based on microwave-assisted extraction (MAE): comparison with QuEChERS method.

Comparison of several extraction procedures for lipid determination in fish.

Fast screening procedure for antibiotics in wastewaters by direct HPLC-DAD analysis

Growing concerns about the contamination of wastewaters by antibiotics, due to human activities, feeding animals as growth promoters or even inefficient wastewater treatment processes, are demanding fast but sensitive analytical methodologies for screening a large number of samples using direct injection of the samples by high performance liquid chromatography with diode array detection (HPLC/DAD), for a multiresidue analysis of 5 antibiotics of different classes. Wastewater from an urban water treatment plant was selected as model to study possible co-elution of interfering compounds. The linearity interval ranged from 40-400 μg L^{-1} for Amoxicillin, Metronidazole, Cefazolin and Chloramphenicol and from 20-200 μg L^{-1} for Sulfamethoxazole, with detection limits lower than 14 μg L^{-1}. This method enables the fast screening of a large number of samples which in the case of a positive detection LC-MS/MS was used. The advantage of the proposed method is to significantly reduce the number of samples to be analysed by more complex methods.

Goal for 2009:

Development of adsorption methodologies using natural materials for antibiotic wastewater treatment

Detection and quantification of antimicrobials in fish and in waters from aquaculture

Electrical sensors are developed for antimicrobials used in cultured fish by potentiometric transduction. Several sensing systems based in ion-exchange or in neutral carriers are setup in a polymeric environment. The influence of the membrane composition and other experimental variables of physical and chemical nature is studied. The main analytical features are reported for devices operating under optimum conditions and the best electrodes selected for further studies.

Automatic methods based on flow injection analysis are established to enhance the routine control of products deriving from the aquaculture section. They are suitable for all analytical laboratories that have moral or legal responsibilities in ensuring food safety to populations, namely industries, fish farms and regulating institutions form each country. These methods are of low cost, easily reconfigured, and enable quick and accurate readings, requiring low amounts of reagents. It is therefore plausible to expect that the proposed methods may constitute good alternatives in a near-future to current official procedures.

The selected sensors are applied to the analyses of samples from aquaculture origin. Antibiotics of tretracyclines and quinolones have been determined in fish and water samples.

This work was developed under contract PTDC/AGR-AAM/68359/2006, initiated in July 2007.

Goal for 2009:

Further antibiotics will be tested in 2009.

Electrochemical determination of antioxidant capacities in flavored waters by DNA biosensors

New biosensors are developed after immobilization and electrooxidation of guanine and adenine as DNA bases on glassy carbon electrode. Square wave voltammetric analysis is used for analytical purposes. The influence of electrochemical pretreatments, nature of supporting

electrolyte, pH, accumulation time and composition of DNA nucleotides on the immobilization effect and the electrochemical mechanism are evaluated.

Both guanine and adenine biosensors are employed for the voltammetric detection of antioxidant capacities of flavored waters. The method relies on monitoring the changes of the intrinsic anodic response of the surface-confined guanine and adenine species, resulting from its interaction with free radicals from Fenton-type reaction in absence and presence of antioxidant. Ascorbic acid is used as standard to evaluate antioxidant capacities of samples. Analytical data is compared with that of FRAP method.

Goal for 2009:

To understand the relation between the antioxidant capacity determined by conventional methods and that of the biosensors, other radical species and antioxidants must be studied. These evaluations will take place in 2009.

Electrochemical Methods in Pesticides Control

Agricultural production currently, and increasingly, depends on the use of pesticides. Pesticide is a term used in a broad sense for chemicals, synthetic, or natural, that are used for the control of insects, fungi, bacteria, weeds, nematodes, rodents, and other pests. These compounds and the products derived from them by degradation or metabolism give rise to residues that may spread through the environment and are particularly frequent contaminants in superficial and groundwaters, in soil and in agricultural and food products.

As many organic compounds used as pesticides contain electroactive groups, voltammetry can be used for their mechanistic and analytical studies. Electrochemical techniques have been very helpful in the elucidation of processes and mechanisms of oxidation and reduction of pesticides. Moreover, the use of electrochemical data combined with spectroscopic studies could provide important information useful to the understanding of the degradation pathways of pesticides in aqueous solutions and in this way to mimicking the environmental processes.

Analytical methods for monitoring the pesticides in environmental samples were developed. Most applications of chemical analysis to pesticide control involve methods with high sensitivity accompanied by sufficient selectivity, precision, and accuracy. Easy sample pre-

treatment and rapid analytical procedures are also desirable. When selecting the method, the cost of the instrumentation and the possibility of performing measurements in the field are also important factors to be considered. Since electrochemical methods satisfy all the above criteria, they were a good choose for the analysis and control of environmental pesticides.

Voltammetry and amperometry have been widely applied to the determination of pesticides in water samples because they require reduced sample preparation and time-consuming extractions steps.

Goal for 2009:

Further pesticides will be analyzed in 2009.

A survey of trace elements in retail samples of flavoured and bottled waters

Macro (Ca, Mg, K, Na), micromineral (Fe, Zn, Cu) and trace elements (Al, As, Cd, Cr, Co, Hg, Mn, Ni, Pb, Se and Si) composition of 39 waters was analysed. Determinations were made by atomic flame, electrothermic atomisation in graphite furnace or hydride generation and cold vapour generation coupled to atomic absorption spectrophotometry.

Mineral contents of still or sparkling natural waters (without flavours) changed from brand to brand. Mann–Whitney test was used to search for significant differences between flavoured and natural waters. For that, the concentration of each mineral was compared to the presence of flavours, preservatives, acidifying agents, fruit juice and/or sweeteners, according to the labelled composition.

Goal for 2009:

Development of a biosensor that will enable the quantification of antioxidant capacity in beverages (flavoured water).

CHEMICAL AND BIOCHEMICAL ENGINEERING

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SOIL REMEDIATION

Industrial activities, intensive agricultural activities and oil industry leaded to the introduction of several types of contaminants in soil that affected soil and surrounding environment quality and can endanger public health. Scientific community has an important role on the development and optimization of remediation technologies that allow reducing the risks of a contaminated site. Following this path, several studies have been developed namely in remediation technologies such as soil vapor extraction (SVE) and bioremediation (BR).

SVE is the most used remediation technology worldwide. It is an in-situ technology that is applicable to soils contaminated with volatile or semi-volatile contaminants such as petroleum products and it uses vacuum pumping to extract contaminated vapors from the unsaturated zone of the soil. Our team has been focused on the evaluation of the impact on the remediation time and process efficiency of some soil properties (contents of organic matter and water), operational parameters (airflow rate) and contaminant properties (vapor pressure). It is also being developed an artificial neural network to the prediction of the remediation time of a SVE process based only on some soil properties and the contaminant involved.

BR is one of the most popular remediation technologies due its low cost. It commonly uses native microorganisms of the soil to degrade the contaminants but can also use inoculated microorganisms that degrade specific contaminants present in soil, creating faster remediations. Our team have isolated and tested several strains that showed high degradative rate of benzene, toluene, trichloroethylene and methyl-tert-butyl-ether in soils even with high concentrations of metals. The combination of BR and SVE is also being performed in order to obtain higher efficiencies that could achieve the legal limits for soil contamination. An artificial neural network capable to predict the remediation time and the efficiency of the combination of these two technologies is the main objective of the project.

This work was developed under contract PTDC/ECM/68056/2006 - Remediation of contaminated soils combining vapour extraction and biological processes: time and efficiency forecasting.

Goal to 2009:

The main objective of this project, and achievable in 2009, is to build an artificial neural network capable to predict the remediation time and the process efficiency of the combination of these two technologies to remediate soils contaminated with benzene, toluene, ethylbenzene and xylene.

Publications:

J.T. Albergaria, M.C.M. Alvim-Ferraz, C. Delerue-Matos, Soil vapor extraction in sandy soils: Influence of airflow rate, Chemosphere 73 (9) (2008) 1557-1561.

V.C. Fernandes, J.T. Albergaria, T. Oliva-Teles, C. Delerue-Matos, P. de Marco, Dual augmentation for aerobic bioremediation of MTBE and TCE pollution in heavy metal-contaminated soil, Biodegradation (2009) .

Sorption of metals using natural materials

Sorption is one of the most used processes for removal of low concentration metals, present in industrial wastewaters. The ion exchange resins and activated carbon are the most used sorbents due to their high efficiency, however the high price limits their applicability. Therefore, research has recently been directed towards alternative adsorbents.

Native natural materials, renewable resources, available in huge quantities in Portugal have been studied as sorbents.

Peanut hulls one of the most abundant food industry waste products was studied for the removal of copper (II), nickel (II) and zinc (II) from aqueous solutions. This work was mainly focused on the following aspects: chemical characterisation of the biosorbent, kinetic studies into a continuous stirred tank adsorber, study of the pH influence in mono-component system, equilibrium isotherms and column studies, both in mono and tri-component systems. All the experimental results were fitted to models. This study was also a preliminary approach to real industrial wastewaters treatment.

Laminaria hyperborea, Bifurcaria bifurcata, Sargassum muticum and *Fucus spiralis* are marine macro algae species abundant at the Portuguese coast that were used for removing toxic metals (Cd(II), Zn(II) and Pb(II)) from aqueous solutions. The Kinetic studies indicated that all the studied macro algae species can provide an efficient and cost-effective technology for eliminating heavy metals from industrial effluents.

Goal to 2009:

Native natural materials, renewable resources, available in huge quantities in Portugal will be tested as sorbents for the removal of metals.

Peanut hulls, one of the most abundant food industry waste products, will be studied for the removal of copper and lead. Equilibrium and kinetic tests will be performed in a batch system both in mono-component systems and in the bi-component system, allowing their comparison.

The marine macro-algae *Ascophyllum nodosum* will be used for copper removal. The effect of the operating variables - temperature, pH and initial concentration - on metal uptake capacity will be studied in batch system. The Box–Behnken factorial design coupled with surface response methodology will be used the mathematical model used.

Publications:

Olga M. M. Freitas, Ramiro, J. E. Martins, Cristina M. Delerue-Matos, Rui A. R. Boaventura, Removal a Cd(II), Zn(II) and Pb(II) from aqueous solutions by brown marine macro algae: kinetic modelling, *Journal of Hazardous Materials*, 153, 493-501 (2008).

Olga Freitas, Cristina Delerue-Matos, Rui Boaventura, Optimization of Cu(II) biosorption onto Ascophyllum nodosum by factorial design methodology, **J. Hazard. Mater. (2009)** (*in press*)

Aurora Silva, Sónia A. Figueiredo, M. Goreti Sales, Cristina Delerue-Matos, Ecotoxicity tests using the green algae Chlorella vulgaris—A useful tool in hazardous effluents management, **J. Hazard. Mater. (2009)** (*in press*)