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POSTERS
ON-LINE COLLECTION/CONCENTRATION FLOW-BASED SYSTEM FOR TRACE METALS USING NEWLY SYNTHESIZED CHITOSAN RESINS POSSESSING CHELATING MOIETIES

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Chitosan, which is a glucosamine biopolymer, is used as one of the useful base materials for the development of chelating resins and ion-exchange resins. It is of great interest due to the advantages, such as easy derivatization of its amino group, and more hydrophilic than the synthetic base materials like polystyrene-divinylbenzene copolymer, which provides fast sorption of ionic species in aquatic media. In this study, chitosan resins modified with chelating moieties were synthesized for the collection and concentration of trace metal ions in aquatic samples for the application to the on-line pretreatment for the flow injection analysis.

Novel chitosan resins possessing chelating moieties were developed by using a cross-linked chitosan resin with EGDE (ethyleneglycoldiglycidylether) as a base material. The adsorption behavior of cationic and anionic species, such as metal ions and oxo-acid anions, on the synthesized chitosan resins was systematically examined by using the proposed ICP-MS measurement coupled with the column pretreatment. Also, we discussed and elucidated the adsorption ability of their chitosan resins for the collection and separation of cationic and anionic species in aquatic media. The chitosan resins could be adsorbed ionic species by the chelating mechanism, ion-exchange mechanism, and hydrogen bonding mechanism.

We present and discuss (1) the synthesis of novel chitosan resins modified with chelating moieties, (2) the adsorption behavior of ionic species on the chitosan resins, and (3) the on-line pretreatment technique for metal ions with chitosan resins.
P002

FULLY AUTOMATED ON-LINE SOLID-PHASE EXTRACTION/LIQUID ELECTRODE PLASMA ATOMIC EMISSION SPECTROMETRIC SYSTEM FOR DETERMINATION OF HEAVY METAL IONS

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In these days, environmental pollution with heavy metals, such as Cd, Pb, Cr, Hg, Cu etc., has been one of big problems to be solved for establishing a safe and reliable society. Now, we can use several spectrometric methods for metal determination, such as atomic absorption spectrometry (AAS), inductively coupled plasma-atomic emission spectrometry (ICP-AES), and inductively coupled plasma-mass spectrometry (ICP-MS). However, such spectrometric methods are a large-scaled and highly expensive analysis system, and less convenient for mobile on-site analysis, which requires a quick/urgent analysis and a less expensive and a less time-consuming analysis.

In recent years, Takamura et al. proposed a new concept of metal detection, and realized it as a portable elemental analyzer, Liquid Electrode Plasma Atomic Emission Spectrometric system (LEP-AES), which is surely a novel atomic emission spectrometry: a small amount (several microliters) of sample solution is put into a micro channel, and a high voltage is applied to the solution from both ends; a micro-plasma is generated in the channel. This technique is much different from a conventional ICP-AES in its principle and instrumentation. The newly developed atomic emission spectrometric system, LEP-AES, is a very compact one: it is easily mobile and very light, and does not need any gaseous carrier. In this work, we propose a
fully computer-controlled on-line analysis system of LEP-AES coupled with a flow-based solid-phase micro-column pretreatment apparatus: Auto-Pret LEPAES system for heavy metal ions in environmental samples.
A novel flow injection analysis system for determination of metal ions with use of alkaline phosphatase (ALP) as a multimetal recognition element was developed.

An apoenzyme reactivation method is catalyzed on mild condition and has high selectivity to cofactor of the enzyme. An ALP is known as a typical metalloenzymes, which has zinc (II) ions as a cofactor and Mg (II) ions for stabilization of its structure. ALP catalyse a $p$-nitrophenyl phosphate (PNPP) to orthophosphate acid and $p$-nitrophenol, and the absorbance of the produced $p$-nitrophenol was monitored continuously at 405 nm by the FIA system.

In this work, we applied this system for determination of zinc (II) ions and magnesium (II) ions. In addition, we applied ALP to determination of cobalt (II) ions. An ALP which has zinc (II) ions as a cofactor has an Asp at position 153 and a Lys at position 328 of the amino acid sequence. On the other hand, ALP which has cobalt (II) ions as a cofactor has a His and Trp at these positions, respectively. Therefore, if we make recombinant ALP which has a cobalt(II) ions as a cofactor from Zn-type ALP, it may enable to determination of cobalt(II) ions. We try to develop determination system for multi metal ions with use of ALP as a recognition element.
P004

MICROFLUIDIC SYSTEM COUPLED WITH ELECTROCHEMICAL DETECTOR FOR DETERMINATION OF METALS

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Miniaturization and automation of analytical instruments for detection of target molecules in situ or in real samples still belong to the main aims of environmental analytical chemistry. Electrochemical techniques are convenient for these purposes because their possibility to be easily miniaturized with low cost and demands on running. Besides the development in control and processing devices, research in the area of miniaturization of electrode system, mainly screen-printed electrodes, is also intensive. These types of electrodes can provide us many advantages such as possibility of mass production, high mechanic and electric durability and possibility of modification of surface with nanostructures to enlarge the detection surface. If screen-printed electrodes are incorporated to microfluidic system with micro electrochemical detection, they provides unique mobile analytical instrument.

The aim of this study was to detect metal ions especially silver using micro and nanoelectrodes connected with miniaturized electrochemical devices and microfluidic system. The electrochemical measurements were carried out in small volume of electrolyte (down to hundreds of µl). There were observed very low concentration levels of silver ions (0 – 0.3 µM). Limit of detection obtained under conditions of time of accumulation 240 s was 10 nM. Electrochemical detection of silver ions on carbon micro and nanoelectrodes provides instrument for ultrasensitive determination of these toxic ions in biological matrices in situ.
REPETITIVE DETERMINATION OF IRON(III) WITH XYLENOL ORANGE USING CIRCULATORY FLOW INJECTION ANALYSIS

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Xylenol Orange (XO) reacts with iron(III) selectively in the strongly acidic solution. The spectrophotometric determination of iron(III) with XO has been carried out by a circulatory flow injection analysis (Cyclic FIA). A cation exchange mini-column for on-line regeneration of the main reagent is incorporated after the detector in the single-line manifold.

A typical reagent solution was made of 500 ml containing $1.0 \times 10^{-4}$ M XO in HCl solution (pH 2.0) and pumped to the single-line FIA system at a flow rate of 1.0 ml min$^{-1}$. Into the stream, an aliquot (100 μl) of a sample containing iron(III) was injected by means of a 6-way valve. The complex formed was monitored spectrophotometrically at 585 nm. Then the reagent stream was passed through a mini-column packed with a cation exchange resin (7 cm×4mm i.d., Amberlite IR124) which was incorporated after the detector. A successful ion-exchange reaction took place, resulting in the accumulation of iron(III) onto the cation exchanger, and the subsequent regeneration of the free XO, which makes the system reversible and the reagent reusable. Then the stream returned to the reservoir.

The excellent repeatability and reproducibility, and the simplicity of this method are well suited for the repetitive iron(III) measurements. The method was successively applied to the determination of iron(III) in environmental waters. The regeneration and recycling of XO reagent allowed as many as 100 repetitive determinations of iron(III) with the same 500 ml circulating solution.
Ascorbate oxidase (ASOD) immobilized onto porous glass beads with controlled pore size (CPG) was packed into a small, plastic column and then, the column was inserted into a temperature probe. The probe was attached to a flow injection calorimetric system (Enzyme Thermistor). Calorimetric microdetermination of copper(II) ions was based on an apoenzyme reactivation method developed by one of the authors. The enzyme activity was calorimetrically monitored by injecting 10.0 mM L-ascorbate solution (pH 5.6). Exposing the minicolumn to \(N,N\)-diethyldithiocarbamate solution (DDTC; pH 8.0) significantly decreased the catalytic activity of the enzyme column. Subsequent injection of 1.0 mM copper(II) ions solution (pH 6.0) led to reactivation of the once deactivated column.

Thus, copper(II) ions in micromolar levels could be calorimetrically determined.
A new catalytic method has been developed for the determination of copper by a FIA system. The method is based on the catalytic effect of copper(II) on the oxidation of 3,3',5,5'-tetramethylbenzidine (TMBZ) in the presence of cumenehydroperoxide (CHPO) as an oxidant. TMBZ is oxidized to a blue compound ($\lambda_{\text{max}} = 650$ nm) at pH 2.9 ~ 3.1 and a temperature of 75 °C. The oxidant solution used was prepared by dissolving in dimethylsulfoxide, because CHPO is sparingly soluble in water. The formation of the blue compound was extremely accelerated by 2,9-dimethyl-1,10-phenanthroline(neocuproine) as an activator. A constant absorbance was obtained over the TMBZ concentration range 3.0 ~ 4.0 mM. A 0.21 M CHPO concentration was selected for the sake of sensitivity and baseline stability. Sampling volume and reaction coil length were selected as 60 μL and 9 m, respectively. By this FIA method, a linear calibration graph for copper(II) was obtained over the range of 0.05 - 0.3 ppb and the reproducibility was satisfactory with a relative standard deviation of 0.2% for 0.1 ppb copper (n = 6). The sampling rate was 30 samples h⁻¹. By using CHPO, the blank values could be kept low. The proposed method was successfully applied to the determination of copper(II) in standard river water samples.
APPLICATION OF EASY AND RAPID COMPLEXATION REACTION
FOR DETERMINATION OF CADMIUM IN SEQUENTIAL
INJECTION SYSTEM

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This contribution presents a new complexation reaction for determination of cadmium in sequential injection system. The reaction is based on the formation of complex between Cd (II), iodide ions and color reagent Quinaldine red. The formed complex causes the shift of absorption maximum from 528 nm for reagent to 630 nm for complex that allows very easy and rapid no separated determination of cadmium. A commercial flow system with eight-way selection valve (FIAlab® 3500) supplemented with the fibre-optic charge-coupled detector USB 2000 and micro-volume Z-flow cell of 20 mm optical path length was used for the optimization of reaction conditions – concentration of iodide ions, dye, wavelength, and operational conditions of system. Under the optimum reaction conditions (60 μL of 5×10⁻² mol L⁻¹ KI; 50 μL of 10⁻³ mol L⁻¹ dye) using 50 μL of sample, the detection limit was 0.43 mg L⁻¹. A frequency of analysis was 20 samples per hour with the relative standard deviation (RSD) 0.69 % (n = 10) at 2.25 mg L⁻¹ concentration level. The method was tested for analysis of model water and pharmaceutical samples spiked by cadmium.

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Sequential injection procedure for determination of organic mercury compounds by using gene modified bacteria

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Elemental mercury and its inorganic and organic compounds are of interest due to their toxicity and impact in our food chain. They play also an important role in environmental issues. Therefore analytical methods for determination of them in biological samples and the possibility to monitor their concentrations in environmental systems are of great importance. In this work we describe a sensitive method for determination of mercury and its compounds based on a biological sensor.

MC10611(pmerRBlux) is a gene modified E.coli bacteria that has been shown to give luminescence when contacted with mercury containing species [1]. Due to the toxicity of mercury compounds and due to the biohazard of the bacteria the solution handling during the assay work should not be done manually. We have developed a sequential flow injection method to prepare samples and to perform the analytical determination to avoid skin contact with the bacteria and the mercury species in the samples. The sequential injection analysis (SIA) protocol requires only small amounts of sample and reagents.

A special flow trough cell to catch and concentrate the bacteria has been designed and constructed. The cell contains a Pt sputtered surface which charge can be controlled to make it to a “bacteria trap”. This enables concentration of the bacteria on a specific and well defined area of a sensitive light sensor.

**P010**

**DETERMINATION OF TOTAL MERCURY IN WHOLE BLOOD BY CHEMICAL VAPOR GENERATION FROM ALKALINE MEDIUM**

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Cold vapor generation (CV) using acid medium and NaBH₄ as a reducing agent (E⁰ = 0.482 V) with detection by atomic absorption spectroscopy (CV-AAS) is the most employed technique for the determination of mercury [1]. However, the reducing power of NaBH₄ in alkaline medium is higher than in acid medium (E⁰ = 1.28 V). Therefore, the use of alkaline medium for the determination of total mercury by CV is a very promising alternative.

In this work a method for the determination of total mercury (methylmercury, MeHg⁺ and inorganic mercury, Hg²⁺) in whole blood was developed. For that, chemical vapor generation (CV) from alkaline medium with detection by atomic absorption spectroscopy (AAS) was used. A continuous flow system (CF) was designed for introduction of sample and reagents. The samples were alkalinized with 0.05 mol L⁻¹ NaOH (pH = 9.25) and the incorporation of antifoam (Foamkill to 0.4% v/v) was necessary. The MeHg⁺ present in samples was mainly transformed to MeHgH with 0.2% m/v of NaBH₄ to be then atomized in an electrically heated quartz cell at 700 ºC. While the inorganic mercury was totally reduced to Hg⁰ using the same NaBH₄ concentration and detected under the same previous conditions. All chemical and continuous flow analysis parameters were optimized. The detection limit (LOD, 3σ) for both species of 0.485 µg L⁻¹ was achieved. The precision of the method, expressed as RSD% for ten successive determinations of a solution of 40 µg L⁻¹ of both mercury species varied within the range of 0.55% to 0.64%. The accuracy of the method was evaluated by means of recovery of organic and inorganic mercury added to the samples, with quantitative recoveries between 91% and 101%. The method was applied to the determination of mercury in 50 samples of whole blood of people occupationally unexposed to the element and the results were compared with those obtained using a previous developed methodology in acid medium.
MICROCOLUMN SORPTION OF ANTIMONY FOR ITS PRECONCENTRATION AND DETERMINATION IN ENVIRONMENTAL SAMPLES BY FI-ETAAS

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Antimony is ubiquitously present in the environment as a result of natural processes and human activities. Antimony is an element of environmental concern due to its toxicity and biological effects. Antimony and its compounds are considered to be priority pollutants interest by the USEPA and the EU. Antimony is a non-essential element in plants, animals and humans and its toxicity is similar to that of arsenic. Around 3.8 x 10^{10} g/year of antimony are released into the environment as a consequence of man's activities. Antimony compounds are employed for industrial purposes (for example, glass, plastics, and ceramics and thermocouple industries) and medical uses. Antimony enters the aquatic environment as a result of the weathering of rocks, from soil runoff, through effluents from mining and manufacturing and from municipal discharge. Antimony is transported in the atmosphere over long distances. Inhalation exposure to antimonials has been reported to produce pneumonitis, fibrosis, bone marrow damage and carcinomas.

The direct determination of total antimony in many environmental samples is very difficult because of the lack of sufficiently sensitive and selective method. Usually obtained detection limits of the spectrometric techniques such as atomic absorption spectrometry and inductively coupled plasma atomic emission spectrometry are insufficient. Because of the extremely low concentrations of Sb in environmental samples a preliminary concentration is usually necessary.

A comparative study about two simple and rapid procedures are described for the reliable determination of antimony in environmental samples based on sorption of Sb chelate in two resin placed in autosampler arm of ETAAS. The results obtained for the determination of antimony in different environmental samples are summarized for the two methods.
Platinum, palladium and rhodium are active components of automobile catalysts, which allow to remove 90% of carbon monoxide, nitrogen oxides and hydrocarbons from exhaust fumes. As a result of abrasion and deterioration of the surface of the catalysts during vehicle operation, these metals are emitted into the environment. It has been shown that there is a clear dependence between the increasing use of catalysts and increasing concentrations of platinum in roadside environment (road dust, tunnel dust, soil, plants, river water and sediments). It has been proven that platinum can be partially dissolved from road dusts, sediments and soil and can enter into a food chain. Hence, the monitoring of platinum content in the environment is necessary. Due to very low concentration levels of Pt in environmental samples (ng/g) and complex matrix composition, the determination of this element requires the use of analytical methods of high sensitivity and selectivity.

In this work Pt was determined by flow-injection chemiluminescence methods (FIA-CL). Determination of Pt was based on the quenching effect of the analyte on chemiluminescence emission generated by lucigenin-NaOH and lucigenin-NaOH-ascorbic acid systems. The developed FI-CL methods of Pt determination are characterized by low limit of detection (0.7 ng/mL) and good precision (RSD < 3%). Unfortunately, these methods suffer from interference of matrix origin since other metal ions and organic compounds can affect the CL emission. In order to increase the selectivity of the methods, column filled with algae *Chlorella vulgaris* immobilized on Cellex-T resin was used for separation of Pt from environmental samples. The quantitative and selective sorption of analyte on algae column was obtained for samples acidified with HCl (pH = 1.6 – 1.8). The application of algae column resulted in increase in the sensitivity of the methods (enrichment factor was 4 in lucigenin-NaOH
system and 9 in lucigenin-NaOH-ascorbic acid system) and in the tolerable concentration of matrix ions in comparison with direct measurements. Increase in selectivity was more pronounced in method based on lucigenin-NaOH reaction, so this method was applied for the determination of platinum in environmental samples (river water, road dust). The critical evaluation of the proposed methods in terms of their advantages, limitations and applicability for determination of Pt is performed in this work.
Rhodium and palladium are mainly used with platinum in automobile catalysts and in catalysts in the chemical industry. The treatment of exhaust gases from motor vehicles equipped with catalytic converters has resulted in the removal of about 90% of carbon monoxide, unburned hydrocarbons, and nitrogen oxides from the exhaust. The dust containing these metals is deposited along the roadways, on soil and vegetation, and in bodies of water. A clear link has been established between the increasing use of automobile catalysts and increasing environmental concentrations of precious metals.

During the first years of automobile catalyst impact research, the focus lay on Pt as the main component of Pt/Rh-catalysts. Pt levels of a variety of environmental matrices are already known. However, corresponding Rh and Pd data are mostly missing.

More often, the choice of the analytical technique depends on the availability and the level of occurrence of the metals and the nature of the sample matrix. Atomic absorption spectrometry (AAS), both flame (FAAS) and electrothermal (ETAAS), has been widely used for the determination of PGEs in various materials. Separation, preconcentration and dissolution of samples are the vital steps in many procedures, owing to the very low concentration of these metals in many samples and the complexity of the matrix. Despite the discontinuous character of the ETAAS technique, many efforts have been made to design procedures which imply on-line pre-concentration of analyte prior to detection. Solid phase extraction (SPE) has been demonstrated to be a very effective pre-concentration method. The SPE process can be carried out either in off-line or on-line mode. In latter case the sorption columns are mostly located at the injection loop or mounted on the tip of the auto-sampler arm.
In this work, an on-line pre-concentration procedure was developed by FI-ETAAS for simultaneous determination of rhodium and palladium.
A new and completely automated multisyringe flow analysis (MSFIA) and multipumping flow analysis (MPFS) system, coupled to a long path length liquid waveguide capillary cell (LWCC), is proposed for the spectrophotometric determination of uranium in different types of environmental sample matrices, without any manual pre-treatment, and achieving high selectivity and sensitivity levels. MSFIA and MPFS are especially suitable for minimization of reagent consumption (green chemistry) and the monitoring of environmental parameters, since reagents are propelled to the system only when necessary and they allow development of fully automated systems with a high injection throughput.

Environmental actinide concentration is usually very low, excepting cases of accident or nuclear power plant releases, so in this work we propose to isolate and preconcentrate uranium by means of a TRU resin. Eicrom’s TRU Resin is an extraction chromatographic material which allows the transuranide separation. After elution, uranium is determined spectrophotometrically after reaction with Arsenazo III. Combination of the MSFIA and MPFS techniques with the TRU-resin allows carrying out the sampling preconcentration and isolation in a short time using large sample volumes.
The proposed FIA (flow injection analysis) is based on a modified Berthelot reaction of ammonium ion with 1-naphthol and dichloroisocyanurate to form an indophenol blue derivative. We choose 1-naphthol as phenol compounds because it reacts with ammonium ion to form the indophenol blue derivative without nitroprusside and is lower toxicity than phenol. Dichloroisocyanurate is used as hypochlorite donor because of a stable powder reagent. We study to improve the sensitivity of the modified Berthelot reaction and propose the addition of acetone, which is a substitute of nitroprusside.

The FIA consists of two or three line flow system. Carrier solution, reagent solution 1, and 2 are used in the FIA. Carrier solution is deionized water. Reagent solution 1 is an alkaline - sodium dichloroisocyanurate solution containing of a complexing reagent. Regent solution 1 is used as a carrier solution when the two line flow system is used. Reagent solution 2 is 1-napthol in mixed solvent (water, acetone, and ethanol). The interference of foreign ions in the sample is studied, for example, nitrite, nitrate, and metal ions. Common anion dose not interfered with the determination of ammonium ion. However, metal ions forming hydroxide interfere because the indophenol blue derivative with Berthelot reaction is formed under high alkaline solution. The interference of these metal ions is removed by the addition of the complexing reagent, such as citrate. The FIA could be applied to river and sea water samples without the pretreatment of distillation or gas diffusion.
A novel detection method for ammonium ions based on a spectro photometric FIA with use of pyocyanine as a coloring reagent in combination with micro fluidic gas diffusion device was developed in this study.

A blue pigment produced by microorganisms was isolated, characterized and then, applied to an FIA (Flow Injection Analysis) as a coloring reagent followed by investigation of the properties.

The pigment was separated by a silica-gel column chromatography, and the molecular weight was determined by DI-MS. The structure was analyzed by $^1$H-NMR. The isolated compound was found to be identical to pyocyanine. The pigment-producing microorganism was identified as Pseudomonas sp. from an 16S DNA analysis. Noting that the property of the pyocyanine of which absorbance depended on the pH shift, the compound as a coloring reagent was applied to an FIA for determination of ammonium ions.
THE DEVELOPMENT OF PERVAPORATOR-FLOW SPECTROPHOTOMETRY FOR ON-LINE CYANIDE SOLID SAMPLE ANALYSIS

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Solid sample analysis frequently involves sample pretreatment including destruction, distillation, or extraction which is laborious, time consuming and consumes large volume of samples and reagents prior to analysis. Therefore, the existence of techniques allowing the on-line analysis is extremely demanded in order to overcome those problems.

The development of on-line system was started by constructing a pervaporator made of stainless steel as the donor chamber and perspex for the acceptor chamber. This donor chamber material is relatively stable with the heating systems and acids corrosion. The in-line heating system was build using a nickeline material coated with silicon tubing and connected to a transformator with variable voltage to provide variable degree of heating. The full on-line system of Pervaporator-Flow Spectrophotometry has been applied for determining cyanide from cassava spectrophotometricaly at 267 nm based on the formation of tetracyanonickelate(II). However, the recovery of cyanide was still 82 % compared to the standard method distillation.

Feasible improvement of the design was disscussed and the proposed system is expected to be the bases of the development of in-line solid samples for other solid samples including foods, medicines, or pesticides.
Halogenated aromatic compounds are important environmental pollutants of soil, water and air. Fluorinated compounds are among these due to their useful applications, such as aerosol propellants, surfactants, refrigerants, plastics, pesticides, plant growth regulators, medicines, adhesives and fire retardants [1]. The improper disposal together with the chemical inertness and hydrophobicity of many of these compounds led to their persistence in the environment; the use of bioreactors as remediation technologies has been increasing recently, fully exploiting the microorganisms’ activity [2]. Bioreactors represent a highly potential technology of biological treatment in the degradation of organic pollutants. Systems with high biomass retention that are extremely promising are rotating biological contactors (RBC). RBCs could be used as a biological post-treatment for polishing of wastewaters contaminated with organic pollutants, including micropollutants. These technologies benefit from a continuous monitoring of the biodegradation process through the quantification of the real time byproducts resulting from the biodegradation of the pollutants.

In this scenario, flow methodologies present a fast, simple, reliable and automated solution, and present an advantageous alternative to gas chromatographic methods.

In this work, a flow system for the potentiometric determination of fluoride for a RBC monitoring is described. The operation conditions of the fluoride electrode in the required dynamic range were studied, using different flow configurations in order to
obtain maximum sensitivity with the simpler assembly. Different arrangements (cascade and wall-jet) for incorporating the (ion-selective electrode) ISE in the manifold were also compared in terms of reproducibility and robustness.

The aim for in-line monitoring also required an extensive interference study due to the use of growth medium in the bioreactor.

The samples were collected from different points of the RBC reactor, centrifuged (3000 rpm), filtrated (0.45 µm) and stored in the freezer. Before analysis, they were unfrozen and directly introduced into the system.

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P019

SIMULTANEOUS DETERMINATION OF NITRATE AND NITRITE
BY SIA-FIA METHOD

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A fully automated procedure based on Sequential Injection Analysis (SIA) and Flow Injection Analysis (FIA) methodology for simultaneous monitoring of nitrate and nitrite in drinking water samples was described. Two channels for simultaneous determinations were used. Nitrate were reduced to nitrite inside cadmium column channel while nitrite were directed to the T-point coupled with peristaltic pump with diazo-coupling reagent. Details of the system will be described. Two approaches – FIA and SIA were combined in the developed method. Using of FIA principles for aspiration, mixing and reaction of nitrite with diazo-coupling reagent increased the sample throughput of the method. No mixing with flow reversal was used. SIA principles and multiposition selection valve were used for sample dividing. Developed method was validated and used for drinking water analysis.

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A STUDY ON THE COLOR DEVELOPMENT CONDITIONS OF MOLYBDENUM BLUE FOR THE SIMULTANEOUS DETERMINATION OF SILICATE AND PHOSPHATE ION BY FIA

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The molybdenum blue method is widely used for the determination of phosphate and silicate ion in water and a number of FIA methods based on molybdenum blue have been reported. Also, it is well known that both of molybdate concentration and acidity influence the formation of molybdenum blue and phosphomolybdenum blue is formed at higher acidity than silicomolybdenum blue. In the absence of antimony (III), silicomolybdenum blue is formed at room temperature, but, the formation rate of phosphomolybdenum blue is very low. The formation rate is increased by addition of antimony (III) and the maximum absorbance is obtained at room temperature. We studied in detail the color development conditions of molybdenum blue for the simultaneous determination of phosphate and silicate ion without antimony (III). The FIA manifold consisted of three flow lines for carrier, acidic molybdate solution and ascorbic solution, reaction coil and flow cell. Water injected sample (0.25 mL), sulfuric acid (1.2 mol/L)-ammonium molybdate tetrahydrate solution (5 g/L), ascorbic acid solution (30 g/L) were flowed to reaction coil (0.5 mm i.d.×3 m, 90 °C) at 1, 2, 4 mL/min respectively and an absorbance of phosphomolybdenum blue formed was measured at 830 nm. When sulfuric acid (0.4 mol/L)-ammonium molybdate tetrahydrate solution (5 g/L) was used, both of phosphomolybdenum blue and silicomolybdenum blue were formed and the absorbance corresponding both of phosphate and silicate ion was measured. Difference of the absorbance on the later procedure and that on the former procedure is corresponded to silicate ion.
P021

SIA SYSTEM WITH POTENTIOMETRIC SENSOR ARRAY DETECTION – APPLICATION TO THE SIMULTANEOUS DETERMINATION OF CATIONS AND ANIONS

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The SIA technique has been proposed recently to add versatility and facilitate automation of calibration tasks when employing electronic tongue systems [1]. This novel detection schemes involve the use of a sensor array, normally with cross-response features among the different sensor used, plus a chemometric data processing tool to extract complex response patterns.

A quite successful approach to develop such automated electronic tongue systems has been the use of all-solid-state potentiometric sensors equipped with differently formulated polymeric PVC membranes. The interference problem, between the primary ions considered and closely related ionic interferences – of a very complex nonlinear nature when the number of species increases – is then tackled with the cross-selectivity features very like in the electronic tongue treatment. Different chemometric treatments can then be employed, for example Partial Least Squares; in our experience, we opted for the use of Artificial Neural Networks (ANNs), more suited to the high non-linearities present [2].

When these ideas are then transferred to their use with automated systems, some extra possibilities appear; apart from the obvious measure in steady-state conditions or with the simple peak height, it is possible to use the complete transient response shown by each sensor after placed in contact with the sample. This advanced dynamic treatment is
facilitated thanks to the high reproducibility offered with the SIA system. The transient can be subsequently reduced to significant features, for example through a Fast Fourier Transform (FFT), and the reduced information taken as departure point for the ANN model.

This approach has been successfully tested, for alkaline ions mixtures in one hand [3], and also for anion mixtures in the other [4]. In this communication, we present the simultaneous SIA multidetermination of both anions (nitrate, chloride) and cations (ammonium, potassium). The 8-sensor array used planar microfabricated structures with standard PVC membranes deposited onto a gold contact [5]. The chemometric treatment used first extracts meaningful data from a FFT transform of each sensor’s transient, and then fits an ANN model for quantitative estimation of each concentration of the four-ions mixture. Thanks to the automation capabilities of the used SIA system, all the preparation of the ca. 100 standards used for the building of the response model could be done with no effort, all the work under computer control.

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REFERENCES

GAS FLOW ANALYSIS BY FLUOROMETRIC FIBER WITH UV-LED EXCITATION DEVICE FOR FORMALDEHYDE VAPOR

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Formaldehyde vapor is one of the harmful and injurious VOCs (volatile organic compounds), and has been reported to develop Sick-house syndrome, especially at the hermetic house. In this work, we have constructed a high-sensitive NADH ($\lambda=340$nm) fluorometric biochemical-sniffer by incorporating fibre-optic device with UV-LED ($\lambda=335$nm) based excitation system and FALDH (formaldehyde dehydrogenase) enzyme membrane into a diaphragm flow cell with gas- and liquid-compartments for the (sub-ppb level) formaldehyde monitoring in the gas phase.

The NADH fluorometric sensor was constructed with an UV-LED ($\lambda=335$nm), a fibre optic spectrometer, and an optical fibre probe. The UV-LED light source and the spectrometer were connected to the optical fibre probe by Y-shaped optical fibre. The FALDH immobilized membrane was attached on the optical fibre probe as the separating diaphragm between the gas- and liquid-compartments in the flow cell. Measurement of the gaseous formaldehyde concentration was carried out by PB (w/ NAD$^+$) rinsing to the liquid compartment with the optical probe. The excitation UV light was conducted to the sensing terminal of the optical fibre probe. Various concentrations of gaseous formaldehyde were supplied from a gas generator to the gas-compartment. The fluorescent signals of NADH, produced by enzymatic reaction of FALDH, were then guided to the spectrometer and recorded using a laptop PC.

The change of fluorescent intensity induced NADH generation was observed by the application of formaldehyde vapour. The peak wavelength of the fluorescence was 491nm. The fluorescent intensity of the bio-sniffer was related to the concentration of the gaseous formaldehyde (lower detection limit: 2.5 ppb). The fluorometric bio-sniffer was possible to monitor the concentration change of formaldehyde vapour with good sensitivity and ultrahigh gas-selectivity.
FLOW INJECTION SPECTROPHOTOMETRIC DETERMINATION OF FORMALDEHYDE USING HYDROXYLAMINE SULFATE AND FERROZINE

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Formaldehyde (HCHO) is a toxic and carcinogenic compound present in both residential indoor and industrial environments. Exposure to HCHO in higher concentration can cause well-known effects as irritation of the eyes and upper respiratory tract, burning sensations in the mucous membrane and so on. To prevent significant sensory irritation in the general population, an air quality guideline value of 0.1 mg/m³ (0.08 ppmv) as a 30-minute average is recommended by the World Health Organization. On the other hand, discharge of HCHO in hydrosphere should be also paid attention. Japanese government partly revised environmental quality standards for water pollution in 2003 [1], and HCHO was added as a monitoring substance in living environment items. The guideline value is ≤ 1 mg L⁻¹ in rivers and lakes. In the present work, we proposed an affordable and manageable flow injection method for the measurement of HCHO in industrial wastewater.

Hydroxylamine sulfate reacts with HCHO to form formaldoxime. In this reaction hydroxylamine decreases proportionally to the concentration of HCHO. The decrease in hydroxylamine

CS₅ & CS₆: carrier solution (H₂O), RS₁: 1×10⁻⁵ M hydroxylamine sulfate, RS₂: 1.6×10⁻⁴ M iron(III) + 8×10⁻⁴ M PDTS + acetate buffer (pH5), V: 6-way valve, P₁ & P₂: double plunger pumps (0.3 ml min⁻¹), H: 90°C, D: spectrophotometer (562 nm), RC₁: 0.5 mm i.d. × 8 m, RC₂: 0.5 mm i.d. × 6 m, R: recorder, W: waste.

Fig. 1 Schematic flow diagram of the FIA system.
can be measured by using the redox reaction with iron(III) in the presence of ferrozine (the trade name is PDTS), i.e. the amount of the produced purple iron(II)-PDTS complex ($\lambda_{\text{max}} = 562$ nm) decreases with increasing HCHO.

In Fig. 1 a scheme of the FIA system can be seen, also the used optimum conditions of the system. Introduction of formaldehyde sample via a 6-way valve was recorded as a negative peak. A calibration curve was obtained in the range of $0 – 250 \mu\text{g L}^{-1}$ HCHO. The detection limit (S/N = 3) was $1.60 \mu\text{g L}^{-1}$. The RSD values were 0.42 and 0.36%, respectively ($n = 4$ each) for the responses at 50 and 250 $\mu\text{g L}^{-1}$ standards. The proposed method was applied to the determination of HCHO in industrial wastewater.

A FAST PROCEDURE TO DETERMINE PERACETIC ACID EMPLOYING A MICROPUMPING MULTICOMMUTATION FLOW SYSTEM

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Peracetic acid (PAA) has been used as a pre-disinfectant in water supply to improve the quality of raw water, reducing the amount of pathogens and the formation of chlorinated byproducts. A rapid analytical procedure is desirable for the analysis of PAA, since its diluted solutions are unstable. A flow procedure based on a multicommitted flow injection analysis process (MCFIA) to determine the PAA was proposed (Fig. 1).

![Flow diagram of the multicommitted flow system. P1, P2 and P3: solenoid micro-pumps; S: sample; R: reagent solution; x: confluence; Det: detector, 565 nm; W: waste.](image)

The method was based on the reaction of DDPD (methyl-substituted form of N, N-diethyl-p-phenylenediamine) with PAA in a medium using a commercial kit Vacu-vials® (CHEMetrics, Inc). Three micro-pumps solenoid were used to insert samples of the carrier solution (water) and reagent (kit Vacu-vials®). This was monitored at 565 nm.
Under optimum experimental conditions, a linear response ranging from 0.5 to 5.0 mg L\(^{-1}\) PAA (R = 0.9991); a RSD of 1.5 % (n = 10) for a sample of 2 mg L\(^{-1}\) PAA; a detection limit 0.02 mg L\(^{-1}\); a sampling throughput was of 36 determinations per hour; reagent consumption of 80 µL per determination (this volume is 25 times smaller than the volume used in the measures on the bench with kit); and a waste generation of 280 µL per determination were achieved.

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P025

DETERMINATION OF ASCORBIC ACID BY REVERSE-FIA METHOD BASED ON THE DEDIAZONIATION REACTION

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A very simple reverse flow injection analysis (rFIA) manifold with spectrophotometric detection was developed for indirect determination of ascorbic acid in pharmaceutical formulations and the result was compared with method reported in the British Pharmacopoeia. Analytical parameters such as stability, accuracy and precision were established for the method and evaluated statistically to assess the applications of the methods. This method was based on the dediazoniation reaction. When ascorbic acid accelerates dediazoniation reaction of diazonium ions, the quantity of derivatization product from coupling unreacted diazonium ions with phenol to give an azo dye (coupling reaction) was decreased in amount equal to the ascorbic acid concentration. The rFIA design was based on the injection of sodium nitrite into acidic $p$-aminoacetophenon carrier stream in which diazonium ions was formed. This ion was inhibited by ascorbic acid line before coupling with phenol-Na$_2$CO$_3$ line. Under optimum conditions ascorbic acid acts in accordance with the Beer law at two concentration ranges 0.4-6.0μg/ml ($R=0.9986$) and 7.0-20.0μg/ml ($R=0.9949$), with detection limits of 0.25μg/ml.
P026

DEVELOPMENT OF A SEQUENTIAL INJECTION SYSTEM FOR THE GLUCOSE MEASUREMENTS BASED ON A FLOW-THROUGH REDOX SENSOR.

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Due to the current demands on developing more sustainable analytical methodologies, the new automatic methods must not only be simple, robust and versatile, but also, guarantee a strong minimisation in wastes generation. In this context the use of flow-through sensors could contribute to the reduction of reagents consumption, provide an enhancement on sensitivity and consequently an improvement in analytical performance.

In the present work a flow-through sensor was assembled to a sequential injection analysis (SIA) system. The sensor was based on the redox state of thionine (oxidized form is colored (blue) and the reduced form is colorless) that was monitored at 621 nm. This redox indicator was immobilized on gel beads and subsequently packed into a flow cell. The use of a SIA manifold allowed a high versatility for promoting chemical reactions before the detection by the sensor. So, the proposed system was used for the determination of glucose after the enzymatic oxidation of the analyte that resulted in the formation of glucono-lactone and NADH. The produced NADH promoted the colour depletion on the surface of the sensor, proportional to the glucose concentration in the sample.

Physical and chemical parameters such as pH, concentration of reagents, temperature, and reagents and sample volumes were studied in order to attain the best analytical performance. The current methodology will be used for the determination of glucose in real samples.

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FULLERENES FOR AROMATIC AND NON-AROMATIC N-NITROSAMINES DISCRIMINATION

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The detection of N-nitrosamines (NAms) in water supplies is an environmental and public health issue because they are classified as probable human carcinogens by the US Environmental Protection Agency. According to the control levels established by this Agency, non-aromatic NAms [maximum admissible concentrations (MACs) ranged between 20-2000 ng L⁻¹] are more toxic than aromatic ones, which MACs have not been established to date except for N-nitrosodiphenylamine (700,000 ng L⁻¹). In recent years the methods developed to determine NAms have been focused on the detection of these compounds at low nanogram per liter levels but no research has been done on the possibility of discriminating between probable carcinogenic or non-carcinogenic NAms.

From that premises, a simple and novel method based on a fast solid-phase extraction unit containing two sequential sorbent columns has been developed to discriminate between the two fractions of NAms according to their toxicity. A sample volume of 25 mL was passed through a C₆₀ fullerene column that acts as a filter retaining only the aromatic fraction via π-π interactions, and then the effluent was passed through a LiChrolut EN column where the non-aromatic fraction (corresponding to N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitrosomorpholine, N-Nitrosopiperidine and N-nitrosopyrrolidine) was retained. Following elution of the non-aromatic NAms with 150 μL of ethyl acetate- acetonitrile (9:1), 1 μL of the extract was injected into a GC/MS. The method allows the quantification of NAms in drinking and wastewaters according to the MACs established (limits of detection of 4-15 ng L⁻¹) without matrix effects plus recoveries near to 100 % . Moreover, the sample-to-sample extraction time is very short (ca. 10 min) whereas the sorbent columns have a long lifetime (6 months), which makes it the more attractive method.
P028

DEVELOPMENT OF A MULTI-PUMPING FLOW SYSTEM FOR CHEMILUMINOMETRIC DETERMINATION OF NICOTINE


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Nicotine is the principal alkaloid in tobacco and is present as a major component of tobacco smoke, being responsible for widespread human use of tobacco products throughout the world because of its addictive properties. This alkaloid is easily absorbed into the bloodstream through the lungs, rapidly distributed throughout the body exerting a number of physiological effects in both active and passive smokers. Nicotine and its metabolite cotinine can be measured in various biological fluids, including blood, saliva and urine. Therefore, these compounds have been widely used as biological markers to determine tobacco smoking status and estimate exposure to environmental tobacco smoke.

In this work a chemiluminometric methodology for determination of nicotine in biological fluids was developed. The presence of nicotine results in an enhancement of the chemiluminescence emitted from oxidation of luminol by hydrogen peroxide. The developed procedure was implemented resorting to the multi-pumping flow concept that uses multiple solenoid actuated micro-pumps which are accountable for solutions insertion, propelling and commutation, conditioning the establishment and subsequent detection of the reaction zone. Multi-pumping flow systems (MPFS) are characterized by a pulsed flow inherent to the micro-pumps actuation that results in a chaotic movement of the solutions in all directions leading to improved mixing conditions and thus to efficient homogenization of the reaction zone. The improved mixing conditions provided by MPFS are particularly attractive for application in situations that required a fast sample/reagent mixing, as in relation to measurements of short-lived CL emissions. The use of solenoid micro-pumps as the only active devices assured a simple, versatile, easily controlled and compact analytical system that provided low reagent consumption and generation of reduced wastes.
PHOTO-CHEMICALLY INDUCED FLUORESCENCE DETERMINATION OF TIGECYCLINE BY A MULTICOMMUTATED FLOW-ANALYSIS ASSEMBLY.


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Tigecycline is a first-in-class glycylcycline intravenous antibiotic, which has shown a broad-spectrum of antibacterial activity against anaerobic bacteria, Gram-negative and Gram-positive. It is mainly used for the treatment of complicated skin and skin structure infections, as long as complicated intra-abdominal infections.

Up to date, only liquid chromatography has been used for the determination of tigecycline, noticing a complete absence of any spectroscopy method in scientific literature (the compound was put on the market on June, 2006). As a result, our main goal has been to develop a simple and inexpensive spectroscopic method for the determination of the analyte.

As tigecycline does not present native fluorescence, its determination has been performed by using photo-chemically induced fluorescence, employing a 30W UV lamp and an irradiation time of 6 minutes; the degradation product is measured at excitation and emission wavelengths of 333 and 453 nm, respectively. The whole multicommutated flow system has been designed making use of three-way solenoid valves, which makes possible its complete automation. This device has been applied to the determination of the analyte in Tygacil®, obtaining identical results to the ones provided by the manufacturer, Wyeth. In addition, a recovery study over this pharmaceutical was performed, showing recoveries close to 100% in all cases.

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Sequential injection analysis (SIA) is a flow technique widely used to automate analytical determinations, with potential application in industrial process control. Typical characteristics of SIA enable automation of diverse operations such as sampling, chemical derivatization, dilution, standard addition, and others, improving the reproducibility, with low sample and reagent consumption and very small waste generation. The typical system is configured in single line by a syringe pump, coupled to a two-way valve that can communicate with the carrier or with a multiport valve through a holding coil where the reaction zone is formed. The detector is placed in a port of multiport valve immediately after the reaction coil. The fluorimetry provides low detection limits and wide concentration range, but the analyte must be fluorescent or a fluorescent derivate must be produced. The alkaloid quinine, originally extracted of the Peruvian quina tree, has diverse medicinal uses as antimalarial, antiparasitic, antiprotozoal, anti-arrhythmic, antispasmodic; in cosmetics to decrease hair loss; in drinks as flavor drinks and bitter digestive aid. But, as alkaloids are toxic if consumed in large doses, the concentration control is mandatory. In acidic medium quinine fluoresces intensely. Determination was performed with excitation in 340 nm (the source of light was an inexpensive LED), measuring the emission in 450 nm (selected with an interference filter) using a photomultiplier as detector. Volumes of sample and reagent were studied to provide minimum dispersion and efficient mixing, a condition which was achieved by aspirating 200 \( \mu \text{L} \) of sample between two 100 \( \mu \text{L} \) of reagent (0.20 mol L\(^{-1}\) H\(_2\)SO\(_4\)) and deionized water as carrier. Flow rate and reaction coil length were 200 \( \mu \text{L} \text{s}^{-1} \) and 50 cm, respectively. Linear responses were observed for quinine sulphate concentrations between 0.020 mg L\(^{-1}\) and 100 mg L\(^{-1}\), described by the
equation: I=(532.2±40.2)+(2.36±0.04)C with R=0.999. The Limits of Detection (LOD) and quantification (LOQ) were 2.25 and 4.51 μg L\textsuperscript{-1}, respectively. The coefficient of variation (n=10) was estimated as 1.9 % at the concentration level of 0.5 mg L\textsuperscript{-1}. The sampling frequency was 60 h\textsuperscript{-1}, consuming 2.13 μL of concentrate H\textsubscript{2}SO\textsubscript{4} per analysis and producing 4 mL of waste. The results obtained by proposed methodology applied to soft drink samples agreed with those obtained by conventional batch fluorimetric method.
CROSS INJECTION ANALYSIS FOR DETERMINATION OF ALBUMIN

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In this work, a valveless technique called ‘Cross Injection Analysis’ (CIA) was adopted for a method development for determination of albumin. CIA platform and the crossing channels were made using mechanical drill. Sample and reagents were introduced into the CIA platform using peristaltic pump via a control box. The analytical method was developed based upon reaction of albumin with tetrabromophenolphthalein ethyl ester (TBPE·H) in the presence of Triton X-100. Absorbance reading of the developed blue product was monitored at 600 nm. Preliminary results have promisingly shown that a linear curve could be obtained in the concentration range of 5 to 30 mg/L albumin (Abs\textsubscript{600 nm} = 0.0099 [albumin] - 0.0431, \( r^2 = 0.991 \)). Discussion will be focused on optimization and the possibility of applying the method to renal dysfunction diagnosis.

Reference

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Control of the total cholesterol level in the body plays an important role for preventing life-style related diseases. The conventional method is troublesome due to the necessity of going to a hospital for invasive blood collection and using the enzyme reaction through many procedures. On the contrary, about 11 percent of the body's cholesterol is found in the skin at the same rate as in the blood, according to the FDA. Therefore, we focus on a simple and non-invasive measurement for cholesterol using a molecularly imprinted self-assembled monolayer (SAM). A gold electrode was immersed in an ethanol solution containing cholesterol and stearylmercaptan, and then washed in ethanol in order to extract the cholesterol as a template molecule. The extraction of cholesterol molecules creates shape-complementary cavities on the SAM, and the detection of electro-inactive cholesterol is achieved using an electrochemical method with potassium ferrocyanide as the redox marker. The change in the oxidation peak current ($I$) shows a linear relationship with the cholesterol concentration. The change of $I$ is related to the cavity concentration for the mass-transport of the redox marker on the molecularly imprinted SAM. When the cholesterol-sensitive SAM recognizes cholesterol, $I$ decreases due to marker diffusion rejection to the gold electrode surface. On the contrary, when the SAM extracts cholesterol, the marker diffuses to the electrode surface and $I$ increases. The sensing properties of the molecularly imprinted SAM, such as sensitivity, selectivity, and reproducibility, have been examined, and it has been applied for simple and speedy electrochemical sensor development.

A purine derivative adenine poses many biological functions. Besides the fact that this molecule is one of the building blocks for RNA and DNA, there are many derivates with their specifics attributes. 2-aminopurine is well known as mutagen. 2,6-diaminopurine is able to replace purine basis in nucleic acids. Benzylaminopurine belongs to phytohormones.

In this study we aimed at study of electrochemical behaviour of derivates of adenine (adenosine-monophosphate, cyclic adenosine monophosphate, adenosine-triphosphate, 2-aminopurine, adenine, nicotinamide adenine dinucleotide, 2,6-diaminopurine, adenosine, 6-benzyl-aminopurine, S-adenosyl-L-Methionine). We measured hydrodynamic voltammograms within the range from 100 to 1,300 mV for all abovementioned analytes. Based on the results obtained, we selected 1,000 mV as suitable for sensitive detection of all derivates. The influences of pH and flow rate on signal height were also tested. pH optimum was 5 and flow rate 0.75 ml.min⁻¹. Dose-response curves were measured within the range from 1 to 100 µM for adenine, 2-aminopurine and for other analytes from 1 to 1,000 µM. Moreover we investigated behaviour of adenine and its derivatives in the presence of two types of matrices. Matrices were human urine and extract of BY-2 tobacco cells. The obtained recoveries for each analytes demonstrated various interaction with these two matrices.
P034

ISOLATION AND DETERMINATION OF CIRCULATING DNA AND SMALL NUCLEIC ACIDS BY PARAMAGNETIC PARTICLES COUPLED WITH MICROFLUIDIC SYSTEM USING MICRO- AND NANOELECTRODES

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Free nucleic acids were firstly detected in blood serum more than seventy years ago. Later on, their relation to tumour diseases was discovered. Small DNA/RNA molecules come into the blood circulation from the cells as a consequence of tumour growth and metastasising. These nucleic acids can give us the information on the mutations present in tumour cells (tumour suppressor genes, e.g. p53) or DNA hypermethylation.

In this study we used paramagnetic microparticles modified with polyclonal antibody recognizing all nucleic acids for DNA isolation. After the isolation the DNA was released from the particles surface and detected by square-wave voltammetry at micro-, nanoelectrodes and hanging mercury drop electrode (HMDE) connected to microfluidic system. The isolated nucleic acids gave well developed symmetric reduction signals at potentials within the range from -1.25 to -1.35 V at HMDE. At the surface of micro- and nanoelectrodes the signals were obtained within the potential range from 0.5 to 1.5 V. The detection limits of circulating DNA were estimated as 1 ng/ml. Plasmatic concentration of free DNA in serum from healthy specimens was varied from 10 to 50 ng/ml. In tumour patients the concentration of free circulating DNA increased up to 100 ng/ml. These results indicate that our micro- and nanoelectrodes can be successfully used for determination of free circulating DNA in blood serum samples after the separation on microparticles.
Sequential injection chromatography (SIC) added the typical characteristics of sequential injection analysis (SIA) to high performance liquid chromatography (HPLC), automating the sampling and injection steps, enabling fast chromatographic separations with low waste generation. The basic system (pressure < 500 psi) is composed by a multiport valve and a bidirectional pump linked by a holding coil. The monolithic C\textsubscript{18} column is placed in a port of multiport valve immediately before the detector. Because many organic compounds have strong absorption of electromagnetic radiation in the UV region, spectrophotometric detection is widely used in chromatographic separations, but provides detection limits that may be considered high, especially for trace analysis in environmental samples. The increase of optical path is a strategy for improvement of detection limits in spectrophotometry. To avoid attenuation of the light beam in long cells, liquid core waveguide (LCW) has been used with a material of refractive index ($n$) lower than that of the internal solution. The cell Type I (only amorphous fluoropolymer Teflon AF2400 $n = 1.29$) has some drawbacks and only few works report its use, but the cell Type II (capillary of silica coated with the Teflon AF2400), which is more resistant
P036

QUANTITATION OF SULFONAMIDES BY A MULTICOMMUTATION FLOW-ANALYSIS ASSEMBLY: USE OF QUENCHING EFFECT ON TERBIUM LUMINESCENCE.

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Sulfonamide drugs are an important class of antimicrobial agents, employed in both medicine and veterinary practice. Although there have been a variety of proposed analytical methods for their determination, many of them are not suitable for routine quality control, due to time-consuming procedures or expensive instrumentation. In this work, we propose a simple methodology, based on the direct quenching effect produced by several sulfonamide compounds on the terbium (III) delayed luminescence. Three sulfonamides have been selected as model analytes: sulfasalazine, sulphanilamide and sulfamethoxazole. The flow assembly has been designed making use of the automatic methodology multicommutation and the proposed method allows the determination of up to 50 samples per hour, making it appropriate for routine quality control in pharmaceutical laboratories. For all the tested compounds, the developed method shows intra-day R.S.D. around 3% and inter-day R.S.D. lower than 6%, being the detection limits approximately 1 mg L⁻¹ in all cases.
A successive determination of cationic and nonionic surfactants using sequential
injection lab-at-valve micro solvent extraction has been developed. The method is based
on ion association formation between cationic and/or K+-nonionic surfactant with
tetrabromophenolphalein ethyl ester (TBPE-H).

For cationic surfactant:

First step: \[ CS^+ + TBPE^\cdot H_0 \leftrightarrow CS^\cdot TBPE^- + H^+ \]

Yellow \[ \rightarrow \text{Blue, } \lambda_{610 \text{ nm}} \]

For nonionic surfactant:

First step: \[ K^+ + \text{Nonion} \leftrightarrow (K\cdot\text{Nonion})^+ \]

Second: \[ (K\cdot\text{Nonion})^+ + TBPE^\cdot H_0 \leftrightarrow (K\cdot\text{Nonion})^\cdot TBPE^- + H^+ \]

Yellow \[ \rightarrow \text{Blue, } \lambda_{610 \text{ nm}} \]

The blue associate is extracted quickly in 1,2-dichloroethane (DCE). Absorbance in
DEC was measured at 610 nm. With addition of KCl, total absorbance of cationic and
nonionic surfactants was obtained. The proposed method provided an automated, novel,
simple and economical strategy for simultaneous determination of cationic and nonionic surfactants without a membrane separator. The extraction takes place in an extraction coil which is set in a SI manifold. The segments are dispensed to a pipet tip (like a miniature separation funnel) fitted onto a port of multi-position valve to be separated. After phase separation, the absorbance of the organic phase (the bottom part in the pipet tip) is measured via optic fibers.

Ion association formation and color development mentioned above were used for the successive determination of cationic and non-ionic surfactants by solvent extraction, and this method has been applied to the determination of cationic surfactant and nonionic surfactants in commercial hair treatment samples.
We studied the air-segmented continuous flow analysis (CFA) for the determination of polyoxyethylene type-nonionic surfactant (NS). The method is based on the extraction of a NS in water sample with toluene, the formation of a complex between a NS and cobalt(II) thiocyanate in toluene phase, and a colored metal chelate between a cobalt(II) in the complex and 4-(2-pyridylazo)resorcinol (PAR) in aqueous phase.

The CFA manifold consisted of flow line for sample, toluene and reagent solutions, reaction coil, phase separator, and flow cell. Toluene and water sample containing NS were flowed to extraction coil (1 mm i.d., 6.24 m) at 0.8, 2.0 mL/min respectively to extract NS into toluene. Then, toluene containing NS and a potassium thiocyanate (5.63 mol/L)-cobalt(II) nitrate (10 mM) mixed solution were flowed to reaction coil (1 mm i.d., 1.04 m) at 0.4, 0.1 mL/min respectively. Finally, toluene containing NS-cobalt(II) thiocyanate complex and PAR solution (1.16 μmol/L, pH9) were flowed to reaction coil (1 mm i.d., 1.04 m) at 0.27, 0.42 mL/min respectively to form the colored metal chelate. Those flows were segmented with air bubbles. An absorbance of cobalt(II)-PAR chelate formed in the aqueous phase was measured at 510 nm. The detection limit of this CFA was 40 nmol/L as heptaoxyethylene dodecyl ether.

We also studied to concentrate a small amount of NS in water sample by using solid phase extraction column. Effects of packing materials, eluents and flow rate of sample on the adsorption and the elution of NS were studied to obtain high concentration rate.
Ionic liquids have been touted as a promising new class of solvent for performing biocatalytic reactions, as they are capable of presenting high enzymatic efficiency, enzyme stability and selectivity, compared to those observed in conventional organic solvents. The feasibility of ionic liquids as solvent media for mediator-assisted reactions catalyzed by tyrosinase was tested using the oxidation of caffeic acid to the corresponding \( o \)-quinone. In the present work a sequential injection analysis system for kinetic studies of the tyrosinase in ionic liquid-containing systems is described. The determination was based on the measurement of the depletion rate of the substrate caffeic acid at its \( \lambda_{\text{max}} \) by the biocatalytic oxidation catalysed by the mushroom tyrosinase. The reaction rates were measured by monitoring absorbance changes at 311 nm during a fixed period (Figure 1). Different 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF4])/buffer mixtures were used. While tyrosinase was active in this water-miscible IL it was also observed its impairment in the presence of increasing concentrations of [bmim][BF4] as an increase of the \( K_M \) was observed while \( V_{\text{max}} \) was kept fairly constant. In addition, a comparative evaluation of the enzyme behaviour revealed that the enzyme activity decreased significantly when the assay was performed in a methanol/buffer mixture as reaction medium. The tyrosinase inhibitory ability of some substrate analogues, such as cinnamic acid and benzoic acid derivatives, was also exploited.

This approach is further pursued by designing a generic tool for performing kinetic studies of enzyme activity and evaluating the effect of potential inhibitors in ionic liquid/buffer mixtures as reaction media.
Figure 1

![Graph showing kinetic measurement of absorbance over time with an inset focusing on the initial stages of the measurement.

Mushroom Tyrosinase + O₂

[BMIm][BF₄]/buffer system

1-Methoxy-2-[(4R,5S,7S,8R,9S)-9-oxa-6,10-dioxo-6,8,9-trihydroxy-4,5,8,9-tetrahydro-5H-pyrido[3,2,1-ij]quinoline-7-yl]ethenecarboxylic acid

1-Methoxy-2-[(4R,5S,7S,8R,9S)-9-oxa-6,10-dioxo-6,8,9-trihydroxy-4,5,8,9-tetrahydro-5H-pyrido[3,2,1-ij]quinoline-7-yl]ethenecarboxylic acid]
MULTI-SYRINGE CHROMATOGRAPHY (MSC) USING A MONOLITHIC COLUMN FOR THE DETERMINATION OF TEXTILE DYES

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The advent of monolithic columns has facilitated the combination of chromatographic techniques with non-separation flow techniques, particularly multi-syringe flow-injection analysis (MSFIA) by virtue of the low overpressure introduced by monolithic columns and has enabled multi-analyte determinations in complex samples by using inexpensive and flexible FIA components.

Azo dyes are the most important dyes in textile industry and sulphonated azo dyes are frequently used and found in wastewaters. These compounds and their degradation products are toxic to the environment for this reason reliable and rapid methods are needed for their determination.

This work presents the MSC determination of some textile dyes (sulphonated azo dyes). A reversed phase C\textsubscript{18} monolithic column with isocratic elution was utilized to separate these compounds and an amine was added in the mobile phase as ion-pair reagent.

To evaluate and optimize the separation of the studied dyes an experimental design was applied. The effect of the main variables (mobile phase, pH, kind and concentration of ion pair reagent…) was studied. The considered responses were the peak resolution and the time of analysis.

The MSC determination demonstrated some advantages in comparison to conventional system, e.g. great flexibility in manifold configuration and the ability to use organic solvents, high sensitivity with low detection limits comparable to those given by other
sophisticated and expensive analytical techniques. If necessary, the use of the multisyringe, enables to perform multi-isocratic chromatographic development (i.e. to use different mobile phases without the need for gradients) or pretreatment of the sample: extraction and preconcentration of sulphonated azo dyes from water samples involving solid phase extraction cartridges, etc.
Quantum dots (QDs), also called semiconductor nanocrystals, show a great potential as fluorescent labels due to their unique optical properties. In particular cadmium telluride QDs have received great attention, in the last decade, for their relatively high optical absorption and for their broad emission colour range. At the same time, new water based synthesis methods were developed, especially for CdTe QDs, aiming at improving their biocompatibility and their utilisation in aqueous media. The analytical utilisation of QDs is somehow restricted to batch systems, although their potential and applicability could be enhanced by resorting to a flow-based methodology, such as a compact fully automated multi-pumping flow system.

Polyamines are extensively studied compounds ubiquitous in microbial, plants and humans where they play important roles in cellular proliferation and metabolism as well as in cancer grow. These compounds are frequently founded in foods, principally in cheese, vegetables and fruit.

This work is based on the capacity of polyamines to complex copper(II) (Cu^{2+}), a strong quencher of CdTe QDs fluorescence. Aqueous QDs synthesis was carried out by using NaHTe as tellurium precursor, and mercaptopropionic acid as capping material. The dots were purified by precipitation and diluted with water (pH = 7.5 by phosphoric acid).

The multipumping flow system was composed by 4 computer controlled solenoid micro-pumps which were used to insert and propelled water, used as a carrier, and Cu^{2+}, polyamine and QDs solutions. Fluorescence measurements were carried out by means of a fibre optic wavelength scanning spectrophotometer at the QDs maximum emission wavelength after excitation at 395nm.
This work presents a convenient way of carrying out three steps of liquid-liquid extraction using a continuous flow system for quantitative analysis of imidazole corrosion inhibitor. The method was developed based on dye-transfer extraction of imidazole with bromocresol purple (BCP) as an ion-pairing agent. Formation and deformation of ion pair was determined by controlling the pH of aqueous phase during extraction with dichloromethane as organic phase. A glass-phase separator was designed and utilized at three sites of the flow system as phase separator. At the aqueous outlet of the last separator, BCP anion was monitored spectrometrically (590 nm) after dissociation of the ion-pairing. Determination of the imidazole corrosion inhibitor was carried out by making a calibration curve, which is a plot between the absorbance and the concentration of imidazole standard. This flow system offers a convenient extraction method and prompt detection with sampling rate of 5 samples h⁻¹. The linearity working range is from 100 to 400 mg L⁻¹. We could successfully apply the method to determine imidazole in oil-field water samples, collected from natural gas pipelines. This method is suitable for routine monitoring of the residual level of the
corrosion inhibitor in gas pipeline. This can help in preventing uneconomical overuse of the inhibitor in petrochemical industry.

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CONTINUOUS SOLID-PHASE EXTRACTION METHOD FOR THE DETERMINATION OF AMINES IN HUMAN URINE USING ON-LINE MICROWAVE ASSISTED HYDROLYSIS

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Human exposure to aromatic amines (AAs) and N-nitrosamines (NAms) may occur in the workplace, through the environment or by endogenous formation within the human body. Due to their toxicity and possible carcinogenicity to humans, the European Union as well as US Environmental Protection Agency has established regulations controlling these amines. Biological monitoring is considered to be the most effective tool for occupational exposure assessment, which occurs mainly by inhalation or dermal absorption into the human body. Thus, AAs and NAms should be monitored in biological fluids of exposed workers and in industrial atmospheres. The development of sensitive, fast and reliable methodologies concerning the determination of amines is mandatory.

It is proposed an on-line microwave assisted hydrolysis-solid-phase extraction method for the simultaneous determination of aliphatic and aromatic NAms, anilines and chloroanilines in human urine by gas chromatography-mass spectrometry, in one analytical run. In this method, conjugated amines are released from urine using a household microwave oven, which provides a high sample throughput (ca. 2 min for the hydrolysis step) compared with conventional acidic hydrolysis (ca. 30-60 min). The simplicity of the developed method makes it very useful for routine analysis; the precision (R.S.D. lower than 6 %) and the detection limits (2 to 26 ng L⁻¹) obtained were satisfactory. The kinetics of amines excretion in the urine of the researchers exposed has been calculated after termination of the exposure to select the sampling time and determine the elimination process. The absorbed dosage was eliminated by 6 h after exposure.
SIMULTANEOUS MEASUREMENTS OF IRON AND CREATININE IN URINE BY CROSS INJECTION ANALYSIS

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Thalassemic patients normally are suffered from the status of iron overload. These patients are often treated with some medicines that will bind with iron for excretion as iron complex during urination. Effectiveness and pharmaco-kinetics of the medication depends on various parameters, and this could be followed by monitoring iron contents excreted in urine. Like other cases of urine analysis, measurement of creatinine is also required at the same time for correction in the analysis.

In this work, we developed a microfluidic flow system, based on ‘Cross Injection Analysis’ or CIA, for simultaneous detections of both iron and creatinine using single wavelength detection. Within the system, two CIA platforms were connected in series before a visible spectrometer (540 nm). Detection principles for iron and creatinine were respectively based on Fe(II)-5-Br-PSAA reaction and Jaffé reaction. For iron, the linear working range is 1-8 mg L⁻¹ (A-A₀ at 540 nm = 4.59 x 10⁻² [Fe(III)] + 8.55 x 10⁻³, r²
For creatinine, the linear working range is 50-800 mg L⁻¹ (A-A₀ at 540 nm = 6.55 x 10⁻⁴ [creatinine] + 2.79 x 10⁻³, r² = 0.999).

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Folic acid (or Vitamin B9) is a water-soluble vitamin involved in a broad variety of biological processes, as long as it acts as an enzymatic cofactor in transference of methyl-group reactions. Folic acid is responsible for the synthesis of DNA bases and DNA chains; therefore, it is essential for the production of new cells, especially during periods of rapid cell division and growth, such as infancy and pregnancy. Accordingly, severe deficiency of folate leads to numerous diseases associated to hindered cell division processes, such as megaloblastic anemia, bone marrow, or fetal diseases (*spina bifida*, neural tube defects, etc.). Folic acid, which is mainly present as folate in physiologically normal conditions, is excreted in urine as more polar catabolites. Therefore, catabolic transformation of folic acid involves its reduction to the tetrahydro form, which is chemically unstable and, thus, easily transformed to *p*-amino-benzoylglutamate. The final product of the folate catabolism is the acetamide derivative of *p*-aminobenzoylglutamate, which is the most abundant folate metabolite in urine and other biofluids, and has no metabolic activity.

An automated method for the analysis of folate and its catabolic products, *p*-aminobenzoylglutamate, and its acetamide derivative in biological biofluids is here presented. This method is based on the on-line hyphenation of a solid-phase extraction step, which is carried out by a Prospekt 2 system, with LC–MS–MS chromatographic separation and detection. The SPE step is performed with MM anion sorbents, by using only 200 µL of sample after minimum sample pretreatment (buffering and centrifugation of samples). The retained analytes are directly eluted with the chromatographic mobile phase to the column, a hydrophilic interaction liquid chromatographic (HILIC)
column. Analysis is performed by positive electrospray ionization tandem mass spectrometry, using multiple reaction monitoring mode (MRM). After optimization of the main variables and validation, the method has been applied to three different types of samples, namely urine, breast milk and blood serum. Although all the target analytes are present in the three types of fluids, the most abundant folate catabolite in excreted biofluids (breast milk and urine) is the acetamide derivative of p-aminobenzoylglutamate. The proposed method is fully automated, rapid and without long sample pre-treatment, which enables its use for routine analysis. Since folate is involved in numerous cellular processes, the metabolic profile of folate catabolites in biological fluids can provide valuable information about the abundance of this essential vitamin in the human body. This can be of especial interest in groups of individuals with increased risk of deficiency (such as neonates or pregnant women) in which a non-invasive analysis is preferred.
AUTOMATIC DETERMINATION OF CATHELICIDIN IN HUMAN SERUM BY ON-LINE SOLID-PHASE EXTRACTION LIQUID CHROMATOGRAPHY–TRIPLE QUADRUPOLE MASS-SPECTROMETRY WITH MULTIPLE REACTION MONITORING

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Cathelicidin, also known as LL-37, is one of the human antimicrobial peptides that take part in the innate immune system, operating in the first line of defense. LL-37 is a peptide with 37 amino acids and with $\alpha$-helix secondary structure. This antimicrobial peptide acts also as chemiattractant of monocytes, neutrophiles and T cellules, and stimulates endothelial prolification. It is synthesized in macrophages, neutrophiles and epithelial cellules. The cathelicidin gen requires the presence of the active form of vitamin D (1,25(OH)$_2$D$_3$) to be activated. This fact makes that people with low levels of vitamin D may present low levels of cathelicidin, and a treatment with vitamin D can increase levels of cathelicidin. It can be of interest to know cathelicidin levels in patients with some disease, mainly unit-care patients, and also to establish normal levels of this peptide in healthy population.

With the aim of providing an appropriate tool for these studies, a fast automated method based on hyphenated solid-phase extraction (SPE) and liquid chromatography–tandem mass spectrometry has been developed and proved to be highly selective and sensitive to determine cathelicidin in serum.

The SPE step was developed using a Prospekt 2 system and Hysphere Resin GP as sorbent, thus providing cleanup/preconcentration of the target analyte in less than 10 minutes. 1 $\mu$l of 10% formic acid solution was added to 100 $\mu$l of human serum, and then, this mixture was diluted ten times with water. 200 $\mu$l of this solution was directly
injected in a Prospekt system. Methanol and 0.1% formic acid were used as solvation and load solvents, respectively. Cathelicidin was retained in the cartridge and then, the initial chromatographic mobile phase (70:30 50% methanol–100% methanol, both with 0.2% acetic acid and 0.1% formic acid as ionizing agents) eluted the peptide to the analytical column (synergy hydro RP 100x2mm, 2.5 µm particle size) for chromatographic separation in 5 minutes. Linear gradients were programmed to obtain 100% methanol at 5 minutes. The column temperature was 40 °C and the total analysis time 15 minutes. The recovery was 96.8%.

Automation of sample preparation by using on-line SPE with automatic valve switching and cartridge exchange has proved an excellent approach for unattended analysis of the target compounds in human serum samples. Minimising human intervention makes the method easy to apply and improves reproducibility and accuracy. In addition to full automation, the proposed approach provides a closed system for maximum protection against degradation and high precision because the number of sample–transfer steps is significantly reduced.
SEQUENTIAL INJECTION CHROMATOGRAPHY (SIC) FOR DETERMINATION OF FAT-SOLUBLE VITAMINS IN HUMAN BLOOD SERUM

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Simultaneous determination of fat-soluble vitamins (A, D2, D3 and E) and metabolite 25-OH D3 by sequential injection chromatography (SIC) in human blood serum was developed. Extraction step included enrichment (5 times) of determined analytes - physiological levels of vitamins in human blood serum are below LOQ of method.

The SIC system (SIChrom™, Fialab®, USA) was equipped with two serial connected monolithic columns Chromolith® RP-18e (Merck®, Germany) – 25 + 5 mm length x 4.6 mm I.D. and 50 mm length x 2 mm I.D. Mobile phase was acetonitrile : water (96:4) and flow rate 0.6 mL min⁻¹. Volume of mobile phase for one analysis was 7.0 mL (2 x 3.5 mL) and volume of injected sample was 30 µL. Detection in UV spectrum was observed at three wavelengths 265 nm (vitamin D2, D3, 25-OH D3), 290 nm (vitamin E, tocol) and 325 nm (vitamin A) separately. Tocol was used as an internal standard and liquid-liquid extraction (into n-Hexan and methanol) was used prior to chromatographic analysis.

In this work for the first time new narrow bore Chromolith® column and two serial connected columns in SIC method were used. Developed SIC method comes from HPLC method used in clinical laboratory.

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ELECTROCHEMICAL DETECTION OF NADH AT CARBON PASTE ELECTRODE MODIFIED WITH VARIOUS METAL OXIDES IN FLOW ARRANGEMENT.

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The detection of nicotinamide adenine dinucleotide (reduced form, NADH) is of great importance in biosensors based on dehydrogenase enzymes, where its oxidized form (NAD⁺) acts as coenzyme. The electrochemical oxidation of NADH requires relatively high overpotential on solid electrodes; therefore the proper redox mediator of electron transfer is usually utilized to enhance the current response [1].

For that reason, the electrocatalytic effect of several metal oxides, namely MnO₂, PdO, PtO₂, Rh₂O₃, RhO₂ and RuO₂, towards amperometric detection of NADH was evaluated. Mediators were incorporated into the carbon paste (5 % w/w), containing graphite and mineral oil as a binder, and packed in groove carbon paste electrode holder [2]. Flow injection analysis with such prepared working electrodes secured in thin-layer flow cell was employed for NADH assays in 0.1 M phosphate buffer (pH 7) carrier solution.

To minimize interference from easily oxidizable compounds the detection potential around 0 V vs. Ag/AgCl is preferred. Highest current response to NADH injections was observed for PtO₂ modified electrodes within this potential region – almost hundred times higher comparing to the bare carbon paste. Among the others, RuO₂ and RhO₂ showed moderate increase only. The relevant calibration curves were ascertained and the detection potential -0.1 V vs. Ag/AgCl was selected as a compromise between sensitivity and repeatability of measurements. Influence from presence of ascorbic and uric acid was also evaluated and possible practical applications were outlined. The use of PtO₂ as a redox mediator show a great potential for construction of amperometric biosensors based on detection of NADH and utilizing dehydrogenase enzymes.
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EVALUATION OF SCAVENGING CAPACITY AGAINST HYDROGEN PEROXIDE IN THE ABSENCE AND PRESENCE OF BIOLOGICAL TARGETS USING A MSFIA-FLUORIMETRIC SYSTEM

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Hydrogen peroxide (H₂O₂), one of the well known reactive oxygen species (ROS), is capable to promote the oxidation of amino acids and also modification of nucleotides. As consequence of these alterations, important functional roles are disturbed and, therefore, a large number of clinical conditions are developed. In this context, research about scavenging activity against H₂O₂ in the presence of biological targets is a feature of considerable interest.

In the present work, the evaluation of scavenging capacity against H₂O₂ in the absence and presence of biological targets was performed using a fluorimetric reaction based on the formation of europium-tetracycline-H₂O₂ complex and automated by multisyringe flow injection analysis (MSFIA). The operational conditions were studied in order to improve the sensitivity of the methodology. The effect of H₂O₂ over cysteine, taurine and adenine, used here as models for biological targets, was assessed and the results showed that H₂O₂ was able to attack all of them. Glutathione and pyruvate, endogenous antioxidant molecules, were then applied as antioxidant model compounds and its scavenging capacity against H₂O₂ was evaluated in reaction media containing (or not) the biological targets. Different response profiles were observed according to the biological target tested.

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SEQUENTIAL INJECTION ANALYSIS BASED ON AN IMMUNO-OPTICAL SENSOR FOR MONITORING OF RECOMBINANT BMP-7 IN A BIOLOGICAL PROCESS.

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A sequential injection analysis (SIA) system was developed to monitor the concentration of recombinant bone morphogenetic protein (rBMP)-7. An optical immuno-sensor was prepared with an optical fiber immobilized with quantum dot (QD)-conjugated antibody. The antibody for the rBMP-7 was first conjugated with CdSe/ZnS QDs via EDC/NHS and then immobilized on the tip of an optical fiber with a diameter of 2 mm. The fluorescence intensity changed with the quantity of samples, which were attached to the QD-conjugated antibody. The system was fully automated using software written in the LabVIEW™ development environment. A number of system variables such as the flow rate of the carrier buffer solution, the binding time, elution buffer etc., were evaluated to increase the sensitivity and performance of the SIA system. The SIA system was employed to monitor the concentration of trehalase on-line in a fermentation process with recombinant Bacillus subtilis.
Fluorescent-based DNA chips have been well-known as conventional large-scale gene analysis tools. However, these chips are currently expensive since their manufacturing requires sophisticated instruments such as hybridization equipments and fluorescent scanners, which has prevented their clinical use and has confined their utilization to research fields. In this regard, we have developed an electrical detection technique based on the change in the resistance of a gold nanoparticle (AuNP) array due to a change in an open bridge structured by hybridization for flow injection analytical system.

The resistance of the open bridge structured AuNP array immediately decreased and became constant in 60 s. The $\Delta R$ value, defined as the difference in the resistance before and after the hybridization, was 100 mΩ with an S/N ratio of over 30. The sensor showed response over a wide concentration range (1 nM-100 μM) with a detection limit of 5.0 fmol. To verify the effectiveness of this system for the identification of DNA mismatches, we carried out experiments using the targeted DNA along with complementary, 1-bp, 2-bp, and 24-bp mismatched (fully mismatched) DNA sequences. The response was the highest for the complementary DNA and decreased with an increase in the number of mismatched bases. Finally, $\Delta R$ hardly changed at the fully mis-matched sequence ($\Delta R < 10$ mΩ). It implies that resistance change by hybridization can be directly detected with a resolution that is sufficiently high for the detection of SNPs.

In this work, an automatic procedure based on a flow-batch approach employing multicommuted flow injection analysis for the determination of amoxicillin in pharmaceutical preparations is described. The method was based on the reaction of, diazotised o-nitroaniline and amoxicillin, in an alkaline medium, producing a yellow compound that was monitored at 435 nm, using a homemade LED-based photometer. The photometer was attached to a reaction chamber, which was made of Teflon.

The flow system manifold comprised solenoid micro-pumps and three-way solenoid valves which were assembled to obtain a compact module in order to minimize waste generation. Under optimum experimental conditions, a linear response ranging from 25.0 to 250.0 mg L\(^{-1}\) (R = 0.9950) amoxicillin was achieved. Other useful features such as a relative standard deviation of 1.3 % (n = 9) for a typical sample containing 100 mg L\(^{-1}\) amoxicillin, a detection limit (3σ criterion) of 0.345 mg L\(^{-1}\), a sampling rate of 16 determinations per hour, a consumption of 400 μL diazo-compound per determination, a waste generation of 960 μL per determination were also achieved. Comparing results with those obtained using a reference method no significant difference at 95% confidence level was observed.

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Concerns regarding the use of drugs with anxiolytic, sedative or hypnotic properties to incapacitate victims and thus facilitate crimes such as rapes, are not a new phenomenon.

The detection of the responsible drug in the biological fluids of the victim is of paramount importance in establishing an effective prosecution. However, the results of the toxicological analysis in these cases are usually negative because of, for example, the delay in reporting, the administration of a single dose and the short half-life of some of the drugs. Thus, the screening and toxicological analysis of drugs in drinks intentionally adulterated is becoming more requested, as it happens more frequently in the cases of Drug-Facilitated Crime (DFC) victims. Drugs, such as benzodiazepines, (diazepam, for example) present anxiolytic, anticonvulsant and CNS depressant effects. These pharmacological effects makes diazepam classified as a DFC drug. Benzodiazepines are drugs that can be easily placed surreptitiously in drinks consumed at entertainment places, such as nightclubs. In this context, the development of portable pulsed flow microsystems for \textit{in situ} and real-time analysis, assume high importance and promising applicability. Multipumping (MPFS) allows implementing compact and portable analytical systems, with high simplicity in automation and control, bringing together all the advantages associated to miniaturization, as for example, the reduction in the solutions consumption, high portability and reduced power requirements.

The aim of this work was to develop an automatic and miniaturized multipumping flow system to detect drink spiking with benzodiazepines. The spectrofluorimetric determination of diazepam through the coupling of a photodegradation unit in the flow system, easily achieved due to the modular nature of MPFS, was implemented. The
photodegradation unit consisted in a UV lamp commercially acquired. The developed flow system is represented in figure 1.

![Flow diagram for diazepam determination](image_url)

Figure 1 – Flow diagram for diazepam determination. $P_1$–$P_2$, solenoid micropumps 10 $\mu$L stroke volume; $X$, confluence point; $R$, reactor coil (2.0 m); $D$, spectrofluorimeter ($\lambda_{ex}=272$ nm, $\lambda_{em}=450$); $L$, Philips UV lamp; $C$, carrier solution (NaOH 0.2 mol L$^{-1}$); $S$, sample (in NaOH, 0.2 mol L$^{-1}$ and SDS, 0.03 mol L$^{-1}$); $W$, waste.

After optimization of all analytical parameters, a linear working response range for diazepam concentrations of up to 40 mg L$^{-1}$ was obtained, with a correlation coefficient of 0.9992. The detection limit was about 1.02 mg L$^{-1}$.

The results obtained for determination of diazepam in five commercial alcoholic beverages by the proposed flow procedure, were compared with the ones obtained through a HPLC procedure, which revealed a good agreement between both methods, with relative deviations comprised between $-1.97$ and $2.05\%$.

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P054

MULTIPUMPING AUTOMATIC PROCEDURE FOR THE CONTROL OF DIAZEPAM IN PHARMACEUTICAL FORMULATIONS.

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All flow analysis methodologies, namely, FIA, SIA, MCFIA, MSFIA and MPFS, have been applied in the chemical control of pharmaceutical formulations. In fact, these analytical methodologies enable the development of automatic systems able to carry out multi-determinations and continuous monitoring, assuring high sample throughput, low reagents consumption and high precision and accuracy, and providing easy to operate compact and portable analytical systems. Multipumping Flow Systems (MPFS) [1] are based on the utilisation of multiple solenoid actuated micropumps for solution insertion, propelling and commutation generating a pulsed flowing stream that influences the establishment and subsequent development of the reaction. The application of multipumping-based methodologies in routine process control is promising as it has been shown in the automation of pharmaceutical analysis using different methods of detection.

This work involves the development of an automatic multipumping flow system for the chemical control of diazepam pharmaceutical formulations, through fluorimetric monitoring after diazepam on-line UV photodegradation. The flow system, comprising 2 micropumps, assured a linear response range up to a diazepam concentration of 40 mg L\(^{-1}\). The detection limit was approximately 0.970 mg L\(^{-1}\). The developed flow system enabled 5 consecutive determinations in about 22 min, of which 15 min where required for exposure of the sample to UV radiation (photodegradation stage).

The developed MPFS manifold proved to be of good analytical value in pharmaceutical determinations being this way an advantageous alternative to other available procedures for the determination of this benzodiazepine in pharmaceutical preparations.

P055

DIRECT DETERMINATION OF CEFADROXIL BY CHEMILUMINESCENCE USING A MULTICOMMUTATED FLOW-THROUGH SENSOR.

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A selective and direct chemiluminescence method has been developed for the determination of cefadroxil, a broad-spectrum antibiotic effective in Gram-positive and Gram-negative bacterial infections. The method involves the implementation of solid-phase spectroscopy in a multicommutated flow system. The anionic-exchanger solid support, Sephadex-QAE A-25, is placed in the flow-through cell, and the reaction between the oxidant reagent (MnO$_4^-$) and cefadroxil takes place on the microbeads, so obtaining the analytical signal. The system showed a linear dynamic range of 2.7-110 µM, with a detection limit of 0.8 µM and a R.S.D. of 3.3% (n=10). In addition, a high sampling frequency, 16 samples per hour, was achieved with the proposed assembly. The analyte was satisfactorily determined in pharmaceutical preparations and human urine, performing a recovery study where recoveries close to 100% were observed in all cases.
P056

DETERMINATION OF NOREPINEPHRINE IN PHARMACEUTICAL PREPARATIONS BY SEQUENTIAL INJECTION ANALYSIS WITH CHEMILUMINESCENCE DETECTION

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Norepinephrine (noradrenaline) (I) is a catecholamine-type neurotransmitter exhibiting sympathomimetic effect. The I is used in the form of bitartrate in the prevention or in therapy of hypotension. The oxidation of I by permanganate in the medium of aqueous H₂SO₄ is accompanied by the emission of chemiluminescence (CL) radiation. The CL signal is enhanced by hexametaphosphate. This CL reaction was used for devising automated sequential injection analysis (SIA) assay of I in pharmaceutical preparations.

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\text{NH}_2
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The PC-controlled SIA setup consisted of a Cavro XL 3000 2.5-ml syringe pump, Vici-Valco 10–port selection valve and Spectra-Physics FS970 flow-through fluorescence detector equipped with a lab-made CL detection module. Optimal order, concentrations and volumes of aspirated zones of reactants were: 44 μl of 25 mM Na hexametaphosphate, 80 μl of I (aqueous test solution), 24 μl of 0.1M H₂SO₄ and 21 μl of 1mM KMnO₄. Calibration curve relating the intensity of CL (peak height) to the concentration of I was linear in the range 1 - 40 μM I; the limit of detection (S/N = 3) was 0.3 μM I. The sample throughput was 96 h⁻¹. The repeatability of the peak heights was characterized by RSD 2.7% or 1.2% for 10 replicate injections of 4.7 μM I or 19 μM I respectively. The SIA-CL method was used for the assay of 1 mg/ml of I in Noradrenalin Léčiva injections.

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P057

DETERMINATION OF INDOMETHACIN AND ITS DEGRADATION PRODUCTS BY SIC WITH GRADIENT ELUTION USING MONOLITHIC COLUMN

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The presented study deals with a new gradient SIC method for the separation and simultaneous determination of indomethacin and its two degradation products - 5-methoxy-2-methylindoleacetic acid and 4-chloro-benzoic acid in topical pharmaceutical formulation. Ketoprofen was used as an internal standard.

Onyx™ Monolithic C18 (25 x 4.6 mm, Phenomenex®) column and a FIAlab® 3000 system (USA) with a six-port selection valve and 5 ml syringe pump were used. Separation was performed using gradient elution and the mobile phase used was acetonitrile and phosphoric acid 0.2%. Different profiles of gradient elution were tested. The optimal conditions were found using a gradient of mobile phase composition 30:70 to 50:50 of acetonitrile to phosphoric acid 0.2 %. Flow rate was 1.2 ml min⁻¹. Individual determination of all mentioned compounds were carried out in 8.5 min for SIC (including all measurement steps) and 8 min for HPLC. Analysis time of separation was 3.5 min (SIC) and 4.5 min (HPLC).

Novel gradient SIC and HPLC techniques with UV spectrophotometric detection were optimized, validated and compared. The chromatographic resolution between peaks was > 2.00 for both methods. Repeatability of retention time (R.S.D.) was found in the range 0.12 - 0.30% for SIC and 0.18 - 0.92% for HPLC, respectively.

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Fast separation of vitamins A acetate (retinol acetate), D3 (cholecalciferol) and E acetate (tocoferol acetate) using short monolithic column in the sequential injection chromatography (SIC) system was described. The SIC system comprised of 5.0 ml syringe pump, 6 port selection valve and fiber optics UV detector with a 10 mm Z-flow through cell. Separation was carried out under following conditions: 20 µl sample volume, column Onyx™ Monolithic C18 (25 x 4.6mm, Phenomenex®), mobile phase acetonitrile / methanol / H₂O 20:20:1 (v/v/v), flow rate 0.9 ml min⁻¹. Detection was observed at three wavelengths 265 nm (D), 290 nm (E) and 325 nm (A).

The optimized method could be applied for analysis of samples containing vitamin D3 with vitamin A acetate; tocoferol acetate could be used as an internal standard in this case. All results were in good agreement with values set by validation authorities – repeatability of retention time (R.S.D.) 0.52% (A), 0.73% (D3) and 0.86% (E); repeatability of peak height (R.S.D.) 1.39%, 0.66% and 0.88%; peak asymmetry 1.44, 1.24 and 1.20; resolution 6.88 (A-D3), 3.33 (D-E). The analysis time was 6 min under optimal conditions. The calibration curves involved 6 experimental points (concentration range 50 - 150 µM), the correlation coefficients were 0.9983 for A acetate and 0.9988 for D3.

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DETERMINATION OF VITAMIN E IN SPIRULINA PLATENSIS HYDROALCOHOLIC EXTRACT BY MEAN OF MULTI-SYRINGE CHROMATOGRAPHY (MSC)

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We present a novel multi-syringe chromatographic system to determine vitamin E as chemical marker in \textit{S. platensis} hydroalcoholic extract. MSC was developed in a Chromolith® Flash RP-18e, (25 mm x 4.6 mm i.d) column using methanol as mobile phase.

We proposed two different procedures for sample treatment. In the first treatment, vitamin E is extracted with hexane, rotoevaporated and redisolved in methanol before injection in MSC system. In this case, multisyringe system in pick-up position loads the sample; later on, a dispense command carried up the sample and the mobile phase to the monolithic column and detector. Residual volume in solenoid valves were considered in sample load.

The second treatment was done on-line, using MSFIA facilities. This second way used a RP-18 disk to retain non-polar metabolites and after that, they were eluted with methanol.

Chromatograms obtained with vitamin E standard were used to establish retention time. And chromatographic parameters as efficiency (HETP=2416 µm), asymmetry factor (As=3.4), and tailing factor (T\textsubscript{USP}=3.1) were evaluated. Similar chromatographic behavior was obtained for vitamin E in \textit{S. platensis} extract and satisfactory resolution was observed. Peak purity, determined by DAD, allows confirming selectivity of this method.

In conclusion: low retention times, satisfactory resolution and economic instrumentation in MSC system offer a new alternative to natural extracts analysis. On-line procedure developed for MSC methodology allows satisfactory determination of vitamin E.

As we have already mentioned in previous works, low instrumental cost, as well as the decrease of time and reagent consumption, allow considering MSC as a good analytical option for simple or complex samples.
**P060**

**DETERMINATION OF ASCORBIC ACID IN PHARMACEUTICAL FORMULATIONS BY REVERSE-FIA METHOD**

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Simple reverse flow injection analysis (rFIA) manifold with chemiluminescence (CL) detection was described and performed for indirect determination of ascorbic acid in pharmaceutical formulations and the result was compared with those of iodimetric titration. Analytical parameters such as stability, accuracy and precision were established for the method and evaluated statistically to assess the applications of the methods. This method is based on the inhibition effect of ascorbic acid on CL intensity generated from in situ formation of Br$_2$ (BrO$_3^-$-HBr) and H$_2$O$_2$-luminol stream, when luminol was injected as a reagent. The calibration curve was linear in concentration range 1.0-16.0µg/ml, with correlation coefficient and detection limit of 0.9959 and 0.4µg/ml, respectively.
SIMULTANEOUS DETERMINATION OF HYDROCHLOROTHIAZIDE AND LOSARTAN POTASSIUM IN TABLETS BY HIGH-PERFORMANCE LOW-PRESSURE CHROMATOGRAPHY USING A MULTI-SYRINGE BURETTE COUPLED TO A MONOLITHIC COLUMN

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This contribution describes use of a separation method based on on-line coupling of a multisyringe flow system with a chromatographic monolithic column for simultaneous determination of hydrochlorothiazide and losartan potassium tablets. The system comprised a multisyringe module, three low-pressure solenoid valves, a monolithic C\textsubscript{18} column (25 mm x 4.6 mm i.d), and a diode-array detector. The mobile phase was 10 mmol L\textsuperscript{-1} potassium dihydrogen phosphate (pH 3.1)-acetonitrile-methanol (65:33:2 v/v/v) at a flow rate 0.8 mL min\textsuperscript{-1}. UV detection was carried out at 226 nm. The multisyringe chromatographic (MSC) method with UV spectrophotometric detection was optimized and validated. Results from validation were very good. The analysis time was about 400 s. The method was found to be applicable to routine analysis of both compounds in tablets. The coupling of the monolithic columns with a multi-syringe flow-injection analysis provides a excellent and inexpensive tool to solve the separation problems without use of HPLC instrumentation.
In the last few years the Bead Injection (BI) principle has been implemented with flow analysis methodologies (such as SIA and conventional FIA) to provide renewable surface flow-through optosensors. The renovation of flow sensing beads in flow-through optosensors is mandatory when the regeneration of their sensing surface can not be appropriately achieved for reasons such as, for example, the impossibility of eluting the species of interest from the beads due to an extraordinarily strong interaction with the solid surface, that is, when the sensing surface is not able to acts reversibly. In such cases, a promising alternative consists of automatically discarding the beads from the flow cell after each measurement and injecting a new appropriate amount of them from a fresh bead suspension.

In this work, the implementation of BI with multicommutation-based flow systems is proposed. A chemiluminescence flow-through sensor is presented upon the use of the well known reaction between luminol and H$_2$O$_2$. Dowex 1x8 luminol-loaded beads were injected in the flow system by means of a six ports rotary valve. Reagents and sample handling was automatically controlled by computer with a multicommutated flow system which used five three-way solenoid valves, a home made electronic interface and a Java-written software. After chemiluminescence signal development, sensing beads were automatically discarded out with a six ports rotary valve without needing to reverse the flow or stop it.

As a model system, the enhancement of the chemiluminescence signal produced by Co(II) on the luminol-H$_2$O$_2$ reaction in alkaline medium is shown for optosensing trace determination of Co(II) and indirect determination of vitamin B$_{12}$. Linear dynamic range
from 1.7 to 50 µg l\(^{-1}\), detection limit of 0.5 µg l\(^{-1}\), RSD of 5.3 %, sampling frequency of 11 h\(^{-1}\) and good selectivity were obtained.

The system can also be used for determining other metal ions and bioanalytes. More research is currently being developed in our laboratory in this sense to expand the analytical potential of these renewable surface chemiluminescence flow sensor system.

P063

A FLOW INJECTION ANALYSIS SYSTEM FOR MONITORING THE FORMATION OF NANOGOLD PARTICLES BY REDUCTION OF HAuCl₄ WITH HYDROGEN PEROXIDE AND THEIRS SEPARATION

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Nanotechnology offers stimulating opportunities for a wide application in the biomedicine. Among the nanomaterials of interest, gold nanoparticles (GNP) have received the maximum attention because of its properties (optical, catalytic, and electrical) that are not only size-dependent but also shape-dependent¹.

Generally, gold nanoparticles are produced by reduction of chloroauric acid HAuCl₄ by various reducing agents such as citric, gallic, and humic acids, hydrogen peroxide, azide and hydrazine¹.

Flow Injection Analysis methodologies have been used for rapid, highly sensitive and routine automatic determination of ionic forms of gold in classical FIA mode in different samples² and to study the catalysis of GNP in some reactions³.

In this work, gold nanoparticles has been synthesized by reduction of chloroauric acid HAuCl₄ by hydrogen peroxide as agent reducer. The classical approach of Flow Injection Analysis with diode array detector has been used to follow the nanoparticles formation and separations of various sizes GNP.

The influence of physical and chemical variables, such as, length of the capillary, flow rate, temperature, concentration and pH of reducing agents, Au(III) concentration, carrier composition, etc., have been optimized.

The morphology, size and shape of nanogold particles has been characterized by using a high resolution techniques as tunneling microscopy (STM) and transmission electron microscopy (TEM).
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References


SYNCRONEOUS DETERMINATION OF CARBONATE AND ORTHOPHOSPHATE BY UTILIZING MEMBRANELESS VAPORIZATION WITH C^4D AND SIMPLE COLORIMETRIC DETECTION

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This work presents a flow system with on-line dual detections for simultaneous determination of carbonate and orthophosphate. Detection of carbonate was carried out by utilizing the membraneless vaporization (MBL-VP) technique. Carbonate was converted to carbon dioxide gas inside the MBL-VP. This resulted in the change in conductivity of the acceptor zone, which could be monitored by using a tubular contactless conductivity detector (C^4D) situated downstream. At the same time, the sample was analyzed for orthophosphate using flow-colorimetric detection based on the molybdenum blue method. The developed method provides rapid and synchronized analyses of the two common parameters required in water analysis. With this method, we could analyze both the anions within 4 min/sample. This method has been successfully applied to determine natural waters. According to the paired $t$-test, our results are not significantly different to the conventional methods at 95% confidence limit ($t_{stat} = 0.842, t_{crit} = 2.145$).

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The determination of phosphate ion at sub-μM level was investigated by applying amplitude modulated flow analysis [1] to a Malachite Green method [2]. The flow rate of sample solution was varied in response to a sinusoidal voltage signal ($V_c$), while that of reagent solution containing ammonium molybdate, Malachite Green and polyvinyl alcohol was held constant. Both solutions were mixed with water (diluent) under holding the total (sample + reagent + diluent) flow rate constant. In a reaction coil (50 cm, 50°C), phosphate reacted with molybdate to form molybdophosphate, and then with Malachite Green (yellow in acidic media) to form green ion-pair. Downstream, the absorbance ($V_d$) of the solution was measured at 625 nm. The amplitude of the wave component that had the same frequency as that of $V_c$ was extracted from $V_d$ signals with a lock-in amplifier. The linearity of the calibration curve in sub-μM to μM range was good ($r^2 > 0.999$). The limit of detection (3.3 σ) was 0.17 μM. The present method is not based on the absorbance value itself but on the amplitude of the fundamental wave component of absorbance. Thus, contrary to conventional flow methods, the present method is much less susceptible to the baseline shift often caused by the adsorption of the ion-pair to the optical window of flow cell.


Amplitude modulated multiplexed flow analysis [1] was applied to the determination of multiple analytes (Fe$^{2+}$ and Fe$^{3+}$) and to the analysis of multiple samples. For the simultaneous determination of Fe$^{2+}$ and Fe$^{3+}$ in a sample, the flow rate of reducing reagent (ascorbic acid) $F_R$ was varied in a triangular fashion, while that of sample solution $F_S$ was held constant. Both solutions were merged with a coloring reagent (1,10-phenanthroline) that was aspirated to the confluence point at the flow rate of $F_T - F_R - F_S$, where $F_T$ is the constant total flow rate. Downstream, the analytical signal of the mixed solution $V_d$ was monitored with a spectrophotometer at 510 nm and analyzed by means of fast Fourier transform (FFT). The concentration of Fe$^{2+}$ was obtained from the direct current component of the signal by taking the positive contribution of Fe$^{3+}$ to the component into account. On the other hand, the concentration of Fe$^{3+}$ was estimated from the amplitudes of the fundamental and higher harmonic wave components. By moving a window for FFT with time, temporal profile of amplitudes was obtained in real-time. The processes were fully automated using a program written in house in Visual BASIC. For the measurement of Fe$^{2+}$ in multiple samples, the flow rates of the sample solutions were independently varied in response to sinusoidal control signals each having different frequency. The solutions were merged with the coloring reagent solution. The amplitudes of the wave components obtained through FFT were closely related to the respective analytes in the sample solutions. As for the determination of total Fe (Fe$^{2+}$ and Fe$^{3+}$) ions, ascorbic acid was added to the coloring reagent prior to the measurement.

STEPWISE INJECTION ANALYSIS OF SOLID-PHASE SAMPLES

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Different methods of flow analysis (flow-injection, sequential injection, zone fluid and cross injection analysis) are generally used for the automation of the chemical analysis of solutions or gases. For the chemical analysis of solid-phase samples is used the schema of analysis that includes a stage of preliminary manual dissolution of samples and flow determination of analyts in solution. So the flow analysis methodology is applicable only for final stage of analysis and don’t permit realization of full automation of similar techniques.

Stepwise injection analysis (SWIA) [1] opens new opportunities for automation of the chemical analysis of solid-phase samples. SWIA assumes the strict reproduction of all stages of the analysis, characteristic for stationary techniques: dissolution solid-phase sample by an intensive stream of gas, addition solutions of reagents, mixing solutions by a stream of gas, thermostatting (if necessary), pause for achievement of the maximal value of an analytical signal (if necessary), and measurement of an analytical signal.

Possibilities of SWIA are demonstrated by examples of water-soluble samples analysis: mineral fertilizers and pharmaceuticals.

METHOD DEVELOPMENT FOR THE DETERMINATION OF TRACE HEAVY METALS BY SEQUENTIAL INJECTION-ANODIC STRIPPING VOLTAMMETRY USING BISMUTH-COATED SCREEN PRINTED CARBON NANOTUBE ELECTRODE

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Developed method for simultaneous on-line determination of Pb(II), Cd(II) and Zn(II) at low µgL⁻¹ concentration levels by the sequential injection-square wave anodic stripping voltammetry (SIA-SWASV) was developed. Bismuth film was prepared by in situ plating of bismuth onto the screen printed carbon nanotube electrode as a working electrode and hydrochloric acid as a supporting electrolyte. Operational parameters such as ratio of carbon nanotubes to carbon ink, bismuth concentration, deposition time and flow rate during preconcentration were optimized. Under the optimum parameters, the linear ranges were 2-100 µgL⁻¹ for Pb(II) and Cd(II), and 12-100 µgL⁻¹ for Zn(II). The limits of detection (S/N=3) were 0.2 µgL⁻¹ for Pb(II), 0.8 µgL⁻¹ for Cd(II) and 11 µgL⁻¹ for Zn(II). High reproducibility was indicated from the relative standard deviations of heavy metal ions were lower than 4% (n=6). The measurement frequency ranged between 10 and 15 stripping cycle h⁻¹. The results indicate that the bismuth-coated screen printed carbon nanotube electrode, combined with SIA, can provide sensitive and robust tool to perform rapid on-line monitoring of trace heavy metals by ASV.
P069

UV-PEDD PHOTOMETRY DEDICATED FOR BIOANALYTICAL USES.

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Detection of p-nitrophenol, a product of several enzymatic reactions, is useful and important in many fields of modern bioanalytics. We have developed and applied a complete photometric system dedicated for such measurements. The instrument consists only of paired emitter–detector diodes (PEDDs) [1] coupled with an extremely simple and low-cost signal transduction system. The detector is extremely economic and easily customized for specific experimental setups. The experimental data confirmed that this photometric system applied under stationary as well as flow conditions of measurements is useful for fast and reproducible determination of alkaline phosphatase (ALP, EC 3.1.3.1) activity.

[1] Ł.Tymecki, Paired Emitter-Detector Diodes (PEDDs) as detectors for flow analysis, Lecture presented at this Conference

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P070

LED-BASED DETECTORS FOR FLUORIMETRIC MEASUREMENTS.

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Light emitting diodes (LEDs) commonly play role of light emitters of nearly monochromatic light. Various compositions of the semiconductor gate of diode lead to diversity of LEDs which can cover a broad spectral range from UV to NIR. Recently, the analytical utility of LEDs as light detectors has been intensively investigated and several devices based on paired emitter–detector diodes (PEDDs) are demonstrated from those for simple absorbance measurements to flow-through photometric detectors dedicated for chromatography as well as for flow analysis [1].

In this contribution the potentiality of LEDs as fluorescence detectors is announced. Simple three-LED based device for simultaneous photometric and fluorimetric detection of quinine as well as flow system based on flow-through fluorimetric PEDDs for determination of fluorescein, calcein and calcium will be presented.

[1] Ł. Tymecki, Paired Emitter-Detector Diodes (PEDDs) as detectors for flow analysis, Lecture presented at this Conference

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INVESTIGATION OF THE DETECTION MECHANISM IN A PAIRED EMITTER-DETECTOR DIODE FLOW ANALYSIS SYSTEM

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The detection mechanism in a Paired Emitter-Detector Diode (PEDD) was investigated to determine a protocol for selecting the optimum detector light emitting diode (LED) wavelength specific to the emitter LED wavelength.

The PEDD has emerged in recent years as a highly sensitive, low cost, miniaturized LED based detector in flow analysis systems including flow injection analysis (FIA) [1] and liquid chromatography (LC) [2]. This colorimetric detector employs two LEDs, operating one as a light source and the other as a light detector. The emitter LED is forward biased and the detector reverse biased. A simple timer circuit measures the time taken for the photocurrent generated by the emitter LED to discharge the detector LED from 5 V (logic 1) to 1.7 V (logic 0). Applying the PEDD to the detection of phosphate [3] and nitrite [1] previously achieved detection limits in the nano-molar range.

A study into the detection mechanism of the detector LED was carried out in an attempt to improve upon the sensitivity already achieved employing the PEDD. Selecting a detector LED of longer wavelength than that of the emitter LED has to date been the only guideline when selecting the detector LED. As such the flow analysis detection systems reported so far have typically employed a red LED (ca. 660 nm) as the detector LED of choice. In the work reported herein, the optimum detector LED wavelength was investigated for emitter LEDs across a broad spectral range (255 – 880 nm). In addition to the effect of detector wavelength on sensitivity, detector LED light intensity was also investigated.
References


An optical detection system combined with an organic light emitting diode (OLED) and an organic photodiode (OPD) is one of the promising detectors for micro-flow analysis using a microchip because of easy fabrication and integration on a single microchip. In our previous report, an OLED with a layered structure, Glass/ITO/TPD/6wt% Ir(ppy)$_3$:CBP/BPhen/Alq$_3$/LiF/Al, which was prepared by a vacuum deposition method, was applied to a light source for sandwich fluoro-immunoassay of IgA on a microchip, where fluorescence of resorufin, the product of enzymatic reaction of Amplex Red with horse radish oxidase labeled on the secondary antibody of IgA was detected by a CCD line camera. In this case, since the emission wavelength is relatively wider, a band-pass filter is necessary for cutting off the longer light overlapped with fluorescence of resorufin. We have fabricated a new OLED which emits light with a narrow wavelength from the edge, taking into account of the fact that the cutoff phenomenon in waveguide occurs when the wavelength of the propagating light is longer than the cutoff wavelength of the waveguide [1].

In this paper, we will present performance of the optical system combined with the newly fabricated OLED with narrow band emission and an OPD with a layered structure, ITO/CuPc/C60/BCP/Ag in a batch system as well as a flow system. We will also present application of the optical system as a fluorescence detector for immunoreactions and enzymatic reactions.

New methods for bioreactor preparations are still needed and investigated. Different support materials (glasses, plastics, etc.) and immobilized biomolecules are applied, in order to obtain bioreactors with particular characteristic which are suitable for specific analytical uses. The main goal of presented research was to develop simple, fast and effective procedure of biomolecule immobilization on plastic tubes to produce open-tubular bioreactor useful for flow analysis. Alkaline phosphatase (ALP, EC 3.1.3.1) was chosen as a model enzyme. Ordinary polyvinyl chloride (PVC) tubes were applied as bioreactor bodies. The enzyme was covalently bound to reactor surface using one-step carbodiimide method. Several protocols for enzyme immobilization were tested. Operational and storage lifetimes as well as dynamics of bioreactors tested under flow conditions are promising.

These investigations were supported by the Polish Ministry of Science and Higher Education (Grant no. N N204 029736)
A SINGLE STANDARD CALIBRATION MODULE FOR FLOW ANALYSIS SYSTEMS BASED ON SOLENOID MICRODEVICES

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Only two computer-controlled microsolenoid devices, namely two micropumps or one micropump and one microvalve, are sufficient for the construction of on-line dilution modules useful in several flow analytical systems for the calibration using single standard. Three simple constructions of such modules were tested and compared. The most promising is the one based on the concept of a microvalve controlling dilution ratio of the standard and a solenoid micropump playing a double role: solution pumping device and mixing segments homogenizer. All investigated modules were tested with paired emitter detector diode (PEDD) [1] as photometric flow-through detector and bromothymol blue as a model analyte. The best module was implemented into more advanced flow-injection system dedicated for optical detection of alkaline phosphatase activity using UV-PEDD-based flow-through detector for the enzyme reaction product.

[1] Ł. Tymecki, Paired Emitter-Detector Diodes (PEDDs) as detectors for flow analysis, Lecture presented at this Conference

These investigations were supported by the Polish Ministry of Science and Higher Education (Grant no. N N204 029636)
EXPLOITING A MULTIPUMPING FLOW SYSTEM FOR MONITORING THE REPRODUCIBLE SYNTHESIS OF CdTe QUANTUM DOTS.

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Over the past several years the research on colloidal semiconductor nanocrystals (quantum dots or QDs) has experienced a huge development and a great widening of the fields of application due to their unique size-dependent photochemical properties related to the fact that the critical band gap can be tuned over a wide energy range without any modification in the chemical composition simply by adjusting particle size. This unique tunable property of QDs makes them attractive tools for application in analytical chemistry, namely in biological and biomedical assays, where they could be used as luminescent biolabels advantageously replacing hazardous radioactive markers or poor-stability fluorescent or chemiluminescent organic dyes, as well multicoloured photoluminescent probes in environmental and pharmaceutical analysis, with enhanced selectivity and sensitivity. Several synthetic approaches have been proposed to prepare QDs. The most common method for synthesizing water-soluble QDs, namely CdTe QDs, is through ligand-exchange. Thiol-containing stabilizing molecules (thioglycerol, thioglycolic acid, 3-mercaptopropionic acid, etc) have been used, where the mercapto group thiolates the nanocrystal surface and the carboxylic acid provides solubility in an aqueous environment.

Although many studies on CdTe QDs synthesis have been focused on the influence of pH, ligands, ionic strength, temperature, etc, the continuous monitoring of the synthesis process has never been carried out. Effectively, whether the reaction conditions, the synthesis process is controlled under a time-based routine that is expected to determine the nanocrystal size and therefore the quantum dots photoluminescence (PL) properties. In this work, a fully automated multi-pumping flow system is used to carry out reagents addition to the reaction vessel, including the preparation of telluride precursor, and to real time continuously monitor the synthesis process by surveying the fluorescence emission wavelength of the formed nanocrystals (λ_ex = 395 nm).
EXPLOITATION OF A SINGLE INTERFACE FLOW SYSTEM FOR ON-LINE AQUEOUS BINARY SYSTEM EXTRACTION

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The exploitation of aqueous binary (two-phase) systems for the pre-concentration and separation of desired analytes has gained a noteworthy importance in recent years. The aqueous binary systems can be formed when a certain water-soluble polymer is dissolved in water together with another kind of hydrophilic polymer or with a given inorganic salt at specific concentrations. The resulting systems are composed of two immiscible phases, which are intrinsically aqueous.

These aqueous binary systems have been successfully used for the separation of biological materials because they essentially have a non-denaturing environment. It has also been shown that the aqueous two-phase partition technique can be suitable for the separation of inorganic compounds and small organic molecules.

Although a number of different water-soluble polymers may be utilized to form aqueous binary systems, polyethylene glycols (PEGs) are mainly used in combination with inorganic salts. These liquid–liquid extraction systems have several unique advantages over traditional solvent extraction systems, such as, PEG is not expensive, it is commercially available and biodegradable, in the formation of extraction system no addition of organic solvents is required, and, several inorganic anions can be used as water-soluble extractants. On the basis of these considerations, the aqueous PEG–inorganic salt binary systems can be considered non-toxic, non-flammable and non-volatile, thus being friendly to the environment.

The evaluation of the analytical potential of Single Interface Flow systems (SIFA) for performing metal ion extraction in aqueous PEG–inorganic salt binary systems is proposed in this work. To our knowledge this is the first time that aqueous binary systems are exploited in flow analysis. For moreover, one of the main advantages of
SIFA systems is that they no longer rely on the utilization of well-defined and compelling sample and reagent volumes, but simply on the establishment of an unique reaction interface where mutual sample and reagent interpenetration occurred, which facilitate system configuration and resulted in enhanced simplicity and operational versatility.

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The toxicity of heavy metals is well known and for this reason governments introduce strict regulations relative to metal discharge in the environment. Toxic metals are not biodegradable and can be accumulated on living tissues causing serious diseases. The elimination of heavy metals in aqueous effluents needs the use of expensive technologies. Recently, certain waste by-products from industrial or agricultural processes have been recognised as new cheap sorbents in the removal/recovery of toxic metals. These materials represent a suitable alternative as compared to conventional sorbents frequently used (ion-exchange resins, activated carbon, etc.). The aim of this study is the valorization of vegetable residues coming from industrial processes as biosorbents for metal ion recovery from aqueous solutions. Among the different vegetable wastes used, grape stalks coming from the wine production resulted in a good sorption system for the elimination and recovery of metal ions as Cu(II), Cd(II), Pb(II), Ni(II), Cr(VI) and Cr(III). These studies have been carried out at laboratory scale, in continuous-flow methodology, by using fixed-bed columns filled with the biomaterial and being the aqueous metal solution pumped upward through the column. Up to now, samples were collected from the column outlet during days or weeks and metal concentration was analyzed at the end of the experiment by FAAS. Frequently, this procedure resulted in not-completed or over-time experiments and not-well defined breakthrough curves. For this reason, a real-time automatic monitoring flow-system based on potentiometric chemical sensors is proposed in the present work.
The automatic system for the on-line monitoring of biosorption processes is formed by a PC, a data acquisition card, a signal conditioning interface, the flow-chemical sensors, peristaltic pumps, solenoid valves, connecting tubes and the Virtual Instrument. Sensors are connected to the analog input of the acquisition card through the interface, allowing for the use of up to sixteen sensors. Virtual Instrumentation is the use of customizable software and modular data acquisition hardware to create measurement systems, managing PC hardware and other devices (DAQ cards, valves, pumps) by means of the computer bus, and performing measurements like a real instrument, according to the user interest.

In this work, we develop different aspects of the biosorption monitoring system. First of all, we present two Virtual Instruments (VIs), developed under the LabVIEW environment, for different control options. The first one permits the manual control of all the components (valves, pumps, sensor channels...), as well as the real-time data acquisition front panel. This VI is mainly for the optimization and sensor initial checking or sensor calibration, previous to the main experiment. The other Virtual Instrument, used for long-term experiments, presents the same characteristics of the other VI, but in this case it manages the complete on-line monitoring system, controlling all the components automatically in a pre-set order fixed by the user. It is necessary to point out that both developed Virtual Instruments let to store, process and analyze the information, displaying on the screen the user's preferences.

The real-time automatic flow-system developed in this work has been applied in the flow-injection potentiometric monitoring of the biosorption of copper (II) ions, taking place in fixed-bed columns filled with grape stalk wastes. Manifold and flow parameters have been optimized; column conditions were kept the same as in previous studies, for comparison purpose. Results indicated similar breakthrough curves as obtained by FAAS, but now they are reconstructed much more accurately. In conclusion, the conjunction Virtual Instrumentation-Flow-injection Potentiometry can be an integrated choice for the automatic monitoring of laboratory or pilot-plant processes, resulting in a single, user-friendly, reliable and expandable instrument.

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INTEGRATION OF A CHLORINE AMPEROMETRIC SENSOR BASED ON RIGID COMPOSITE MATERIALS IN A LTCC MICROSYS TEM

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Amongst the several materials used in analytical chemistry with miniaturization purposes, low-temperature cofired ceramics (LTTC) are becoming a good alternative. This is due to some interesting advantages, such as low cost (mass production), reduction of samples and reagents consumption, higher analysis frequency, compact design and portability. Moreover, the used multilayer methodology facilitates the integration of materials of different nature, such as Pt, screen-printed conductors and rigid conductor composite materials.

In this work, a working electrode based on rigid composite material (Epoxy-MWCNT) was integrated in LTTC devices. The reference and counter electrode were integrated following the methodology established in our group [1]. Moreover, the standard solutions were prepared by means of micro-valve (NRSearch), which generates less background noise to the analytical systems.

The system was evaluated and applied to the analysis of environmental parameters, such as chlorine in water solution. The LTTC/epoxy-MWCNT system showed low detection limits and high sensitivity. Moreover, the consumption of sample and reagents was considerably low.

Finally, the benefit of using this approach in terms of analytical performance was demonstrated by carrying out the successful analysis of chlorine in complex matrix coming from a swimming pool.

P079

PERFORMANCE ASSESSMENT OF MOLECULARLY IMPRINTED POLYMER BASED SENSORS BY USING FLOW INJECTION TECHNIQUES

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The FIA techniques can effectively be utilized for applications other than chemical analysis. One such example is sensor development, in which FIA techniques can be exploited to assess its performance, such as selectivity and sensitivity, simply by monitoring a series of responses upon injections of several different interfering components or targeted analyte in different concentrations. We report here some results of such studies on sensors based on molecularly imprinted polymer (MIP) developed in our laboratory.

We have been studying MIP based amperometric sensors, which allows the detection of trace concentrations of adenosine triphosphate (ATP), aiming at food safety applications. The sensor has a small carbon electrode covered with an overoxidized polypyrrole film imprinted with an ATP molecule. The sensor operated in the triple pulse amperometric mode and was characterized in both the batchwise and flow-injection measurements. We have detected trace levels of ATP down to 5 nM without employing any separate preconcentration techniques.

![Fig. 1](image1.png)

**Fig. 1** The flow cell and sensor chip (left-bottom corner) used in this study.

![Fig. 2](image2.png)

**Fig. 2** Preparation scheme of oPPy(ATP): (a) Oxidative polymerization of pyrrole with ATP as a dopant on an electrode, (b) Prepared polypyrrole film doped with ATP, and (c) overoxidation of the polypyrrole film and the creation of the cavity complementary by dedoping ATP.
Molecular imprinting is a technique utilizing tailor-made networked polymers for the recognition of specific analyte molecules, and the polymers can be classified as artificial enzyme systems. Fig. 2 shows the synthetic scheme for the MIP sensor film used here; the polypyrrole film was grown electrochemically on a carbon electrode from a pyrrole solution containing dopant, ATP. The dopant, which is automatically included into the polymer matrix, served as a template of the recognition site. The resulting film (b) was oxidized again (overoxidized) in a basic media to exclude the dopant, which occurred due to removal of the positive charge in the polypyrrole matrix (c). The cavity left upon dedoping is therefore expected to have shape complementarity to the dopant (ATP). The site created by overoxidation usually has high selectivity towards recognition of a template molecule (dopant) [1-3].

Sensitive detection with thin film-based amperometric sensors requires effective removal/concentration of targeted analytes in the sensor films. To achieve this we have adopted triple pulse amperometry and successfully detected ATP of nM levels (Fig 3). We also confirmed excellent selectivity of the oPPy film in the flow injection studies. The ratio of ATP to AMP in the peak height was as high as 16 at the overoxidized polypyrrole electrode, while it was only 1.1 at a bare electrode (AMP, adenosine monophosphate) [4].

References

P080

DETERMINATION OF 2-ETHYLHEXYL 4-(DIMETHYLAMINO)BENZOATE USING FLOW-CELL FOR MEMBRANE-ASSISTED LIQUID-LIQUID EXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRIC DETECTION.

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A flow-cell for microporous membrane liquid-liquid extraction with a sheet membrane was used to extract 2-ethylhexyl 4-(dimethylamino)benzoate (EDB) from urine of solar-cream users and spiked wine samples. The cell enabled the target analyte to be extracted from 7.9 mL of donor solution into 200 μL of acceptor solution (decane). After extraction the acceptor solution was transferred to a micro-vial for GC-MS analysis without derivation.

In this work variables affecting the enrichment factor were also studied, such as organic solvent, extraction time, recirculation flow of the donor solution through the donor chamber, presence of potassium chloride and ethanol in the donor solution, and pH.

The method has been evaluated in terms of linearity, sensitivity, precision, limits of detection and quantification, and extraction efficiency. Limits of quantification were 1 and 3 μg L⁻¹ EDB for urine and wine, respectively. Quantitative analysis has been carried out by applying the method of standard additions. Within-day and between-day relative standard deviation were lower than 12 and 20 %, respectively. EDB was found in the urine of users of cream containing EDB in the concentration interval 1.2 – 7.2 μg L⁻¹. Therefore, this provides evidence of EDB dermal absorption and subsequent excretion through the urinary tract. EDB was not found in the analysed wine samples.

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P081

STRENGTHS AND WEAKNESSES OF FOUR TYPES OF PHOTOMETRIC FLOW-CELLS USED FOR FLOW ANALYSIS

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A study of the performance of four different types of photometric flow-cells used in flow analysis is described. The cells studied were a conventional Z-style cell, mirror-coated single and multi-reflective cells, and a newly designed total-internal-reflection cell. The optical and hydrodynamic characteristics of these cells (optical pathlength, transmittance and signal to noise ratio, hydrodynamic dispersion and susceptibility to schlieren effects) have been evaluated using a series of dye studies. Ray-tracing predictions of the optical pathlength of multi-reflective cells have also been compared with experimental data, and have been shown to agree to within ca. 15%. A comparison of the use of these cells in the determination of molybdate reactive phosphate species in estuarine waters is also reported.

The application of aluminium-coated reflective quartz cells for the direct UV detection of nitrate produced by on-line digestion in the determination of total nitrogen is also discussed. The presence of high concentrations of residual oxidising reagents represents a major interference in the detection of nitrate, and derivative UV spectrometry has been investigated as a means of overcoming this problem.
P082

CONSTRUCTION OF A NEW FLOW-THROUGH CELL FOR SCREEN PRINTED ELECTRODES.

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The advantage of flow analysis, low quantity of reagents and samples and high frequency of analysis, combined with the low cost and high sensitivity of electrochemical techniques, make possible the determination of a very low concentration of heavy metals in an On-Line Multi-Syringe Flow Injection Analysis system (MS-FIA).

A new electrochemical flow-through cell design is proposed and used in this work. It can be use with dissimilar kind of Screen Printed Electrode (SPE), with different thickness. It system allows an easy change of the sensors, important point because of the frequently limited life time of the SPE.

In this work it’s describe the On-line MS-FIA system coupled with the home made electrochemical flow cell designed, used to determined Cd and Pb by Square Wave Anodic Stripping Voltammetry, using a Bi-film Graphite Carbon Screen Printed Electrode as a electrochemical sensor.
Integrated calibration method – developed several years ago – combines in one analytical procedure both the interpolative and extrapolative calibration methods. By this means it gives a chance to: a) verify analytical results in terms of accuracy, b) detect the interference effects in a sample, and c) find the adequate way to eliminate the interference effect and to obtain the results of better accuracy.

Several approaches for realization of integrated calibration have been hitherto developed. The most effective one is so called ‘complementary dilution method’ (CDM) [1]. It can be performed manually but different flow techniques can be also used to realize it in order to make the calibration procedure simpler and faster, as well as to improve precision of analytical results.

It is shown how CDM can be performed by continuous flow, flow-injection and sequential-injection techniques. The instrumental systems dedicated to each of flow modes are presented and operation of each of them is explained. They were used to analysis of synthetic samples and compared with each other in terms of precision and accuracy of the results obtained as well as of consumption of time and reagents.

MATHEMATICAL MODELING OF FLOW INJECTION ANALYSIS

ANALYTICAL SIGNAL

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The flow analysis techniques have been subject of evolution from the classical flow injection analysis to multicommutation and recently to multi-pumping and single interface flow analysis. However, the theoretical knowledge about the systems has not accompanied closely the experimental counterpart developments, being this the major drawback for these new techniques. In practice, the optimization and development of flow based applications is still based on a trial and error process which is far from being the desirable situation.

In this communication we present a mathematical model for the prediction of the analytical signal in flow injection analysis, which we regard as a first step on the development of a general mathematical framework for different flow analysis methodologies. The model is based on an analytical solution for the axial dispersion plug flow model [1]. The axial dispersion coefficients have been determined by non-linear regression, from the experimental UV-VIS spectrophotometric results of a colored solution, using a Levenberg-Marquardt algorithm. The axial dispersion coefficients are described as a function of the known physical parameters of the system (e.g. reactor length and internal diameter) and experimental conditions (e.g. flow rate), which renders the model with a large range of applicability on the optimization of systems with different characteristics.

P085

SINGLE INTERFACE FLOW ANALYSIS (SIFA) MODELING AND OPTIMIZATION WITH CHEMOMETRICS METHODS

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This work proposes the optimization of the recently developed fluid management methodology Single Interface Flow Analysis (SIFA) using chemometrics modelling. Differing from the traditional flow techniques, SIFA no longer relies on the utilisation of definite sample and reagent volumes but on the establishment of a single interface being reaction development dependent of the adjoining solutions mutual inter-dispersion. The influence of the most important physical parameters of a SIFA system on the generated analytical signals was evaluated with linear (multivariate linear regression) and non-linear (feed-forward neural networks) models. A D-optimal experimental design built with three reactor coil properties (length, configuration and internal diameter), flow-cell volume and flow rate, was created to assess the impact of these parameters on two analytical signal properties: maximum slope and interface width. Bromocresol green was used as the dye solution and the analytical signals were monitored by using an optical fibre wavelength scanning spectrophotometer. Results demonstrate that the linear and non-linear models were able to estimate both analytical signal properties with validation correlation coefficients higher than 0.96. No significant differences were observed between the linear and non-linear models in terms of models accuracy and precision.
THOROUGH CHEMOMETRIC OPTIMIZATION OF SEQUENTIAL INJECTION ANALYSIS

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Although chemometrics is more powerful than univariate, the frontal approach has not been sufficiently utilized yet for optimizing sequential injection analysis (SIA) methods. Moreover, in previous chemometrically optimized SIA methods, such effective experimental conditions as sample and reagent volumes were not considered.

This communication presents, for the first time, a more comprehensive chemometric optimization study for developing a SIA method, which was applied for diclofenac assay in tablets, injection and gel formulations. The method was based on a spectrophotometric measurement of the reduction of acidified permanganate by diclofenac. Besides reagent concentrations and flow rate, reagent and sample volumes were subjected to chemometric optimization. The $2^6$ full factorial design was adopted. Main and interaction factors controlling the SIA method were examined.

It has been found that the main factors with their effect types, i.e. positive or negative, were ordered as follows: (+ sample volume) $>$ (- flow rate) $\approx$ (+ permanganate concentration) $\gg$ (+ permanganate volume) $\approx$ (- acid volume). Additionally, no significant interaction effect from less effective conditions, i.e. permanganate and acid volumes, were recorded. It could be concluded that factorial design is a useful approach for screening the effect of experimental conditions on method performance while such chemometric approaches as response surface and simplex are useful for subsequent optimization.
Acknowledgments

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P087

TOTAL ANTIOXIDANT DETERMINATION OF SOME PLANT EXTRACTS, WINES AND FRUIT JUICES BY FI CHEMILUMINESCENCE SPECTROMETRY USING LUMINOL AND CO(II) CATALYST IN THE PRESENCE OF A CHELATING AGENT

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It was developed a FI assembly for chemiluminescence determination of antioxidant activity (Figure 1).

![Figure 1](image)

**Figure 1.** Experimental set-up for FI-CL determination

(a) carrier solution (borate buffer, pH = 9); (b) H₂O₂ solution; (c) Co(II)/EDTA/luminol solution; P – peristaltic pump; I – injection valve; L – mixing tube; C - flow cell; S – sample; PMT – photomultiplier tube; R – computer; W – waste

The sample is injected into a carrier of borate buffer which meet a flux of hydrogen peroxide and Co(II)/EDTA/luminol solution in the chemiluminescence flow cell.

By pumping the solutions through channels (a), (b) and (c) of the assembly it was recorded a high chemiluminescence signal. At the injection of an antioxidant sample in flux (a) the recorded chemiluminescence signal was decreased in accordance with concentration and activity of the antioxidant.

It were studied and optimized the analytical parameters of the method. The developed method was applied for the determination of antioxidant capacity of some plant extracts, wines and fruit juices.
SIMULTANEOUS DETERMINATION OF TARTARIC ACID AND POTASSIUM IN WINES USING A DIALYSIS MULTICOMMUTED FLOW SYSTEM

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Precipitation of the tartaric salts as potassium bitartrate and calcium tartrate continues to be the main cause of physical instability in bottled wines. Prevention of this precipitation is required, since the presence of these crystals in wines can indicate poor quality or even spoilage of the product for the consumer. Thus, the development of simple and rapid procedures to monitor tartaric acid and potassium during the wine stabilization process may constitute a useful approach for wineries.

In this work, a multicommuted flow injection system is presented for the simultaneous determination of tartaric acid and potassium in wine samples. A dialysis unit was introduced in the manifold to minimize possible matrix interferences. Tartaric acid determination was based on the spectrophotometric detection of the coloured complex formed between the diffused analyte and vanadate, in acidic medium. Potassium was monitored using a tubular ion-selective potassium electrode placed in the donor stream.

The proposed methodology allowed determination of tartaric acid and potassium within concentration ranges of 1.00 - 5.00 g L⁻¹ and 390 - 1955 mg L⁻¹, respectively. A determination frequency of 54 h⁻¹ was achieved, with relative standard deviations less than 2.07 and 2.40% for tartaric acid and potassium, respectively. The system was applied to the determination of 20 table and 10 port wines and the results were in agreement with the comparison procedures.

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PHOTOCHEMICALLY-INDUCED FLUORESCENCE DETERMINATION OF RESVERATROL IN WINES BY A MULTICOMMUTATED FLOW-THROUGH OPTOSENSOR.

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Resveratrol is a stilbene produced by plants in response to fungal infection or abiotic stresses, e.g. produced by heavy metal ions. Among other plants, resveratrol occurs in mulberries, peanuts and grapes. Grapes contain a large amount of different phenolic compounds in skins, pulp and seeds, that are partially extracted during winemaking. The determination of phenolic compounds in wine is very important since this group of substances is responsible for several sensorial characteristics, such as colour, flavour, astringency and hardness of wine. There is a broad range in the concentration of resveratrol in different wines. Intervals from 660 to less than 0.68 μg mL⁻¹, and from 100 to less than 0.23 μg mL⁻¹, within red and white wines, respectively, have been reported. Here we propose a flow-through optosensor for resveratrol determination. The assembly is designed using three-way solenoid valves for handling solutions and photochemically-induced fluorescence (PIF) is used as detection technique. An anionic-exchanger, Sephadex QAE A-25, is employed as solid support, being the optimum wavelengths on the solid phase 268/368 nm.
The use of the solid support produces an enhancement on both selectivity and sensitivity, due to the retention/preconcentration of the analyte on the microbeads.
There are few continuous methods in the literature to determine iron, most of them based on flow injection using derivatization reactions with thioctionate, phenanthroline or hydroxylamine and photometric detection, or derivatization with pioverdin with fluorimetric detection. For the determination of Fe(II) and Fe(III) ions in all matrices, including wine, several techniques have been involved including photometric and fluorimetric methods, atomic absorption and electrochemical methods.

In this work, a simple reverse flow injection analysis (rFIA) procedure has been developed for the determination of Fe(II) and total inorganic iron Fe(II + III) in wine samples, natural or after addition of ascorbic acid, respectively. It is based on a reagent-buffer injection into the flowing wine sample, where Fe(II) ions form a complex with dPKPH, in the reaction coil. This chelate (Fe dPKPH, 1:1) shows a green-blue absorption ($\lambda_{\text{max}}=700$ nm), which is used as analytical signal. A Jenway 6300 spectrophotometer was used with a 1-cm flow cell.

Firstly, a batch procedure was developed. This way, a systematic variation of the parameters controlling the formation of the complex in wine was studied. The potential interferences caused by several ions present in wine were evaluated. Secondly, the developed analytical procedure was applied to the reverse flow injection analysis of iron in wine. The influence of several variables on the absorbance of the Fe-dPKPH complex (reagent-buffer, concentrations, injected volumes, reaction coil length and flow-rate) was studied in order to optimize the method. The obtained data were used to construct calibration plots that were used to quantify the Fe(II) in commercial white wines. The calibration graphs used were found to provide similar results than the standard addition method. The proposed rFIA method is accurate, precise and very selective.
P091

USE OF THE LEN’S EFFECT IN SPECTROMETRIC FLOW ANALYSIS FOR QUANTITATIVE ANALYSIS OF SUGAR IN BEVERAGES

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This work presents utilization of the Len’s effect that we normally avoid in flow analysis, when UV-VIS detection is used as detector. The Len’s effect or so-called ‘Schlieren effect’ can cause high blank signals due to difference in the refractive indices between the inserted liquid plug(s) and the liquid carrier. This could result in either positive or negative signals of blank when a spectrometer or similar means of detection is used. Here we could successfully adopt the Len’s effect phenomena to develop an extremely undemanding flow system with a low-cost colorimeter, for quantitative and accurate analysis of sugar in beverages. For sucrose, linear calibration was obtained from 4 to 60 % (w/v) sucrose ($r^2 = 0.993$), with fine reproducibility (3.2 %RSD). The detection limit (3S/N) was 3.17 % (w/v) sucrose. This method was effectively applicable to colored and colorless beverages.

Acknowledgements

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Tea is one of the most frequently used drinks throughout the world. The extracts from tea leaves (Camellia sinensis L.) contain a number of catechin compounds which are structurally primarily flavanols. The main naturally occurring catechins in tea are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC). These compounds have been shown to possess strong antioxidant activity and protect the body from oxidative damage caused by free radicals. Research shows that consumption of tea have been linked to a numerous health benefits, including the prevention of certain tumor growth, diabetes and heart diseases. Catechins are peculiar to green and white tea because the fermentation process during manufacturing black tea reduces the amount of catechins.

The aim of the study was to develop a new method for the determination of catechins by means of high performance liquid chromatography (HPLC) with post-column luminol-I₂ based chemiluminescence (CL) detection. The separation was performed with an isocratic elution using a mixture of methanol and 0,1% phosphoric acid. When the antioxidants were eluted from the HPLC column strong inhibiting peaks corresponding to each analyte appeared on the chromatogram. The CL reaction was compatible with the mobile phase of HPLC and allows the simultaneous determination of three catechins (epigallocatechin gallate, epicatechin gallate and epicatechin) in diluted tea extracts in the range of: 1.0 – 22.0 μg·mL⁻¹ (EGCG), 0.2 – 3.0 μg·mL⁻¹ (ECG) and 0.2 – 0.9 μg·mL⁻¹ (EC). The detection limits were: 0.14 μg·mL⁻¹ (EGCG), 0.11 μg·mL⁻¹ (ECG) and 0.03 μg·mL⁻¹ (EC). The relative standard deviations for four measurements of each compound were in the range of 1,29 – 2,13%. The proposed HPLC-CL method has been successfully applied to determine the content of catechins in a variety of tea products.
Analytical application of soluble manganese(IV) as a chemiluminescence reagent was first reported in 2001 [1]. The oxidant was prepared according to a method of Jáky et al. [2] which was slightly modified. Freshly precipitated manganese dioxide was ultrasonicated for 30 minutes with 3 mol·L⁻¹ phosphoric acid and then left to stand overnight. In our laboratory we found that such prepared reagent is stable for about two months, after that time the quantum yield of chemiluminescence decreased significantly. The highest emission of light was observed between thirteenth and twenty fifth day after its preparation.

To date, most analytical applications of soluble manganese(IV) involve the determination of various organic compounds in simple matrices (pharmaceutical preparations) using FIA methodology. Only few methods enable detection of analytes in more complex samples (biological fluids) after their previous separation from interfering matrix.

To the best of our knowledge, there are no reports of utilizing the chemiluminescence of manganese(IV) for the determination of phenolic compounds (e.g. flavonoids, phenolic acids) which are commonly found in plants and plant derived food and are known to posses strong antioxidative activity. We found that polyphenols generate chemiluminescence upon mixing with acidic soluble manganese(IV) in the presence of formaldehyde. Based on this finding, a new flow injection chemiluminescence method (FIA-CL) was developed for the determination of the total phenolic content (expressed as milligram of gallic acid equivalent per liter of drink) in a variety of plant derived beverages (such as wine, tea, fruit juices). The proposed method offers many
advantages including very low limit of detection (0.02 ng·mL⁻¹), wide linear dynamic range (0.5 – 400 ng·mL⁻¹) and high sample throughput (247 samples·h⁻¹). The relative standard deviation for 15 determinations was 3.8% for 2 ng·mL⁻¹ and 0.45% for 10 ng·mL⁻¹ of gallic acid. A comparison between FIA-CL method and other methodologies widely used in food and beverage industry for the estimation of the total phenol/antioxidant levels (UV absorbance measurement at 280 nm, Folin-Ciocalteu method, 1,1-diphenyl-2-picryl-hydrazyl free radical scavenging method) revealed a good correlation. However, FIA-CL method is much more simple, robust and accurate.


Lactate concentration and beta-lactamase activity in milk samples were studied with enzyme thermistor (ET) technology. Determination of lactate in 1:1 diluted milk samples was performed using a conventional ET device coupled with immobilized lactate oxidase/catalase column. The sample volume of 250 μl and flow rate of 0.75 ml/min were used, and 1:1 diluted milk samples were applied to the studies. The linear response from 0.0625 mM to 1 mM and detection resolution of 0.0625 mM were obtained. The life time of the enzyme column could last for over 30 days with 82.5% of the original activity remaining.

In order to develop a novel method for fast determination of beta-lactamase in milk, detection of penicillinase activity in milk was performed using a modified enzyme thermistor instrument. Unlike the lactate analysis, in this study the immobilized enzymes were excluded from the enzyme column. Instead, an empty column was used as a reactor for mixing substrate (penicillin) and milk samples. The penicillinase activity was detected by measuring the resulting heat from enzymatic cleavage of β-lactam ring which was catalyzed by free penicillinase contained in the milk. In the tests of 1:1 diluted milk samples, calibration curves between 0.25 Unit/ml (penicillinase concentration at 0.7 μg/ml) and 5 Unit/ml (penicillinase concentration at 14 μg/ml) were obtained by plotting the penicillinase activity of milk sample versus the thermometric peak height. The sensitivity of the response was optimized using 0.5 ml/min flow rate and 350 μl sample volume. For both analyses, the effects of flow-rate, sample volume and milk content on thermometric response were also investigated. The response time of enzyme thermistor determination was approximately 6 min per sample.
In this manuscript, a downscaled multicommutated flow injection analysis setup for photometric determination is described. The setup was designed assembling together the flow system module and a LED based photometer, thus the overall inner volume of the flow system was about 160 µL. The usefulness of the proposed setup was proved employing them to develop an analytical procedure for the photometric determination of iodate in table salt using N,N’-diethyl-p-phenylenediamine (DPD) as chromogenic reagent. Accuracy was accessed by applying the paired t-test between results obtained using the proposed procedure and a reference method and no significant difference at 95 % confidence level was observed. Other profitable features such as a low reagent consumption of 7.3 µg DPD per determination; a linear response ranging from 0.02 up to 3.0 mgL⁻¹ IO₃⁻; a relative standard deviation of 0.9 % (n = 11) for samples containing 0.5 mgL⁻¹ IO₃⁻; a detection limit of 2.6 µgL⁻¹ IO₃⁻; a sampling throughput of 117 determination per hour; and a waste generation 600 µL per determination were also achieved.

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Ochratoxin A and aflatoxins are highly toxic mycotoxins produced by Aspergillus species growing in a wide range of food and animal feedstuffs. The European Commission set the maximum level for Aflatoxin B1 (AFB1) in foods to 2 ppb, but new limits are likely to be established at 1 ppb and for Ochratoxin A (OTA) the maximum level in wine is, also, 2 ppb.

The common methods for mycotoxins detection include thin-layer chromatography (TLC), ELISA techniques and high performance liquid chromatography (HPLC). These methods typically require skilled operators, extensive sample pretreatment and expensive equipment. The aim of our work was to demonstrate that a previous developed flow injection immunoassay (FI-IA) system for Aflatoxin M1 (AFM1) [1] can be applied, also, for OTA and AFB1 determination.

The FI-IA system is based on a protein G column inserted in the flow injection system. The first step consists in an incubation of the sample containing OTA / AFB1 (Ag) with fixed amounts of anti- OTA / AFB1 antibody (Ab) and of the tracer (Ag*, OTA / AFB1 covalently coupled to HRP) until equilibrium is reached. In this mixture a competition occurs between Ag and Ag* for the Ab. The mixture is then injected into a flow system where the separation of the free tracer (Ag*) and the antibody-bound tracer (AbAg*) is performed in a column with immobilized Protein G. The antigen – antibody complexes are retained in the column due to the high affinity of the Protein G for the antibody. The
activity of the eluted enzyme label is then amperometrically detected, using \( \text{H}_2\text{O}_2 \) and \( 3,3',5,5' \)-tetramethylbenzidine (TMB).

The immunoassay was optimised relative to conditions for antibody-antigen reaction (antibody concentration, incubation time, temperature, extraction solvent concentration influence) and enzymatic label detection. The results were confirmed by HPLC and SPR (Surface Plasmon Resonance) determinations.


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A MINIATURIZED MULTI-SYRINGE FLOW INJECTION-BASED METHOD FOR AUTOMATED EXTRACTION AND PURIFICATION OF THE VEGETAL PROTEIN RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE/OXYGENASE (RUBISCO)

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In this communication, a novel miniaturized flow-through method based on the implementation of anion-exchange solid-phase extraction into a multi-syringe flow setup is for the first time proposed for isolation of the vegetal protein ribulose bisphosphate carboxylase/oxygenase (Rubisco) from Triticum aestivum. The method capitalizes on the retention of the target protein onto Q-Sepharose strong anion-exchanger packed in a cylindrical microcolumn (10 cm long, 4 mm i.d.) followed by a stepwise ionic-strength gradient elution to stripping out potential interfering molecules (0-0.8 mol/L NaCl) and quantitatively collect Rubisco in a minute eluate volume. The flow manifold is furnished downstream with a diode-array detector for in-line monitoring of the eluate at the unspecific wavelength of 280 nm in order to detect the elution of Rubisco and concomitant extract components. The entire flow system is refrigerated at 4°C including the autocollector of eluate fractions.

A comprehensive investigation of the effect of distinct concentration gradients on the purification process and experimental conditions (e.g., resin amount, column dimensions and mobile-phase flow rate) on column capacity and analyte breakthrough is to be effected. The assembled set-up is aimed at the expeditious evaluation of the efficiency of preliminary operations (e.g., polyethylene glycol precipitation, ammonium sulphate precipitation and the usage of sucrose gradient) commonly endorsed in protocols for cleaning-up of interfering compounds (e.g., pigments and other low-molecular weight compounds) in crude plant extracts prior to anion-exchange separation of Rubisco.
The potential coupling in the flow system of a tandem column configuration embracing anion-exchange separation followed by size-exclusion is to be also assessed for automatic removal of electrolytes in the eluate.

The protein obtained following this methodological approach reaches purity standards as for further kinetic characterisation of the enzyme.
SI-LAB-ON-VALVE ANALYSIS OF HISTAMINE USING POTENTIOMETRIC DETECTION FOR FOOD QUALITY CONTROL

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Miniaturization of ion-selective electrodes provides adequate coupling to systems based on the lab-on-valve concept without meaningful loss of the common use advantages. These aspects are evidenced in this work after appropriate implementation of a miniaturized histamine ion-selective electrode for control in biological materials. The solid-contact electrode is based on a polymeric membrane incorporating respectively α-cyclodextrin as ionophore, 2-fluorophenyl 2-nitrophényl ether as plasticizer and potassium tetrakis (p-chlorophenyl) borate as ionic additive and simply screwed up on the central Lab-on-valve block to achieve a robust coupling. The conventionally shaped histamine electrode responded to histamine double cation in the pH operational range of 3.5-5.5 with a slope of 31.7 ± 1.3 mV dec⁻¹, and with a practical detection limit of (1.6 ± 0.2)×10⁻⁶ molL⁻¹. The miniaturization of the above-described electrode enabled its use as detector in a sequential-injection lab-on-valve system (1), yet with a useful lifetime shortened from 10 months to approximately 1 month under continuous operation. The optimized flow conditions were achieved for sample injection volumes of 70µL propelled towards the detection cell at the flow rate of 12µL s⁻¹ during 20 s followed by a flow rate of 15µL s⁻¹ during 50 s. The potentiometric analysis of histamine in different kinds of fish used as samples furnished results statistically similar to those provided by the chromatographic method. The low cost of the analysis, the speed of the method, the use of low volumes of solutions and use of non pollutant reagents justify the use of potentiometry as an alternative analytical technique for routine analysis of products for human consumption.

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DETERMINATION OF SUDAN I-IV IN CHILLI PRODUCTS USING AUTOMATED ON-LINE SOLID PHASE EXTRACTION COUPLED WITH FLOW-BASED TECHNIQUE-MASS SPECTROMETRY

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Synthetic Sudan dyes (I-IV) are widely used as coloring agents in chemical industries. For many years they had been also widely employed as an additive in foods, particularly in chilli powder. However, now its use as food additives is prohibited worldwide, for its cancerogenity. In this work, a rapid, sensitive and on-line solid phase extraction-liquid chromatography-mass spectrometry (SPE-LC-MS) method was developed for determination of Sudan I-IV in chilli products. Two HPLC pumps were used, one for isocratic elution and the other for gradient elution. The analytes were enriched in SPE column and were back-flushed out from SPE column connected to HPLC analytical column (C18, 2.1x150 mm. 3µm) using column switching. Sudan dyes were eluted by the gradient elution programming (0.1% formic acid aqueous solution/0.1% formic acid acetonitrile) following the MS analysis. The limits of detection and the limits of quantitation for all substances were 0.03 and 0.05 µg/g, respectively. Chilli powder were fortified with sudan dyes at LOQ level, the recoveries ranged from 94.56 to 99.90% (R.S.D. ranging from 0.050 to 0.195%). It was found that total analysis cycle time was 12 minutes per sample. The on-line SPE-LC-MS method allows the possibility for the determination of these dyes within a short time. The other advantages of this developed method are increasing sensitivity and reducing amounts of sample and solvent used.
Peroxidase (E.C. 1.11.1.7) is an enzyme commonly found in vegetables that can be responsible for a negative effect on the colour and on the flavour of raw or processed food. Its activity is directly implicated in the enzymatic browning of the vegetables. To extend the shelf life of vegetables they can be submitted to a blanching process. This process is a thermal procedure designed to inactivate the enzymes responsible for the generation of off-flavours and off-odours. Since peroxidase appears to be the most heat stable enzyme in plants, the assessment of its remaining activity is widely used to evaluate the effectiveness of the food thermal blanching. It is generally accepted that, if the peroxidase originally present in the sample is destroyed, then it is quite unlikely that other enzyme systems have survived.

The determination of peroxidase activity has been described based on colorimetric, chemiluminescence, electrochemical or fluorimetric detection of the product formed from the peroxidase reducing substrate. In this work, a spectrophotometric detection of the peroxidase activity in vegetables extracts is described using a flow method with sequential injection lab-on-valve format. The system is based on the reaction between hydrogen peroxide (H$_2$O$_2$) and 2,2-azino-bis(3-ethylbenzothiazoline-6) sulphonic acid (ABTS) catalysed by the enzyme (HRP). The method presented a low sample consumption of 15 µL and low consumption of ABTS and H$_2$O$_2$ of 24 µg and 12 µg respectively, per assay. It was possible to achieve a linear range up to 2 mg/L with a throughput of 1 determination per minute, which corresponds to an increase on the determination rate of 50% comparatively to the comparison method. It was also possible to monitor the on-line thermal inactivation of peroxidase at different temperatures.
Susana Vidigal thanks Fundação para a Ciência e a Tecnologia (FCT) and FSE (III Quadro Comunitário) for the grant SFRH/BD/23040/2005.
A method based on on-line solid phase extraction (SPE) coupling to high-performance liquid chromatography (HPLC) for the determination of sulfonamides has been developed. An SPE procedure was automated to perform sample clean-up and extraction into a homemade microcolumn connected to a sequential injection analysis (SIA) manifold. A simple SIA manifold included one syringe pump and two multi-position valve was constructed. This method can continuously perform extraction of sulfonamides from aqueous samples, which can then be analyzed by HPLC with electrochemical detector. One milliliter diluted sulfonamides was injected into a conditioned SPE microcolumn and the matrix was washed out with water. Sulfonamides were eluted by methanol and transferred to the analytical column by the chromatographic mobile phase. The conditions for on-line SPE, including reagent flow rate, ratio of sulfonamides to mobile phase and time for eluting sample were also optimized. The results of reagent flow rate was 8 µL, ratio of methanol to mobile phase was 90:10 and time for eluting sample was 20-24 second.
FLOW ANALYSIS TECHNIQUES AS EFFECTIVE TOOLS FOR SIMPLIFIED AND IMPROVED ENVIRONMENTAL ANALYSIS OF ORGANIC COMPOUNDS AS TOTAL INDICES.

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The scope of this work is the accomplishment of an overview about the current state-of-the-art of flow analysis techniques applied to the environmental determination of organic compounds as total indices. These techniques are proposed as effective tools for the quick obtention of preliminary chemical information about the occurrence of organic compounds in environmental analysis prior the use of more complex, time-consuming and expensive instrumental techniques. Recently proposed flow analysis based innovations have produced improved methodologies for this type of parameters, and overcoat for the determination of chemical oxygen demand, halogenated organic compounds and phenolic index.

The aim of the present work is highlight flow-based techniques as vanguard tools in environmental analysis of organic compounds, specially emphasizing how Multisyringe Flow Injection Analysis (MSFIA) technique can allow us the accomplishment of fast determinations of Halogenated Organic Compounds expressed as Adsorbable Organic Halogens (AOX).
THE MERCURY DETERMINATION IN AIR BY ELECTROCHEMICAL AUTOMATED METHOD
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The stripping voltammetry is a widely used method of mercury determination in the atmosphere, one of the most sensitive and easily automated methods of the analysis. As a result, there is a big scientific interest in the development of flow analysis with stripping voltammetry (SVA) detection for the quantitative determination of mercury in the environment.

In the present work a new technique of cyclic injection determination of mercury in the air was developed, it includes absorption of mercury from air into the extent of the background electrolyte (0.1 M HCl + 1 M HClO4 + 1·10^-4 M HNO3) and iodine alcohol solution (1·10^-6 M). The analysis was carried out directly in the reactionary vessel of hydraulic system of cyclic injection analyzer with the subsequent stripping voltammetry determination of the analyte in the absorbing solution.

With the purpose of mercury determination in absorbate, a combined electrochemical standardless method was used. The principles of stripping voltammetry and potentiostatic coulometry were included in this method. The presented scheme of electrochemical experiment includes three consecutive stages of stripping voltammetry measurements, in each mercury was accumulated on the electrode at different times Δt1, Δt2, Δt3 according to the following conditions: \((\Delta t_2 - \Delta t_1) = (\Delta t_3 - \Delta t_2)\). After each stage of preconcentration anode dissolution of a deposit from the electrode was carried out with the registration of the peak of mercury dissolution \(E_p= 0.65 \pm 0.05\) В.

The values of quantity of electricity \(Q_1\), \(Q_2\) and \(Q_3\) were found by integration of registered currents on time. Quantity of electricity \(Q_\infty\) was calculated from L. Meites’s equation.

\[
Q_\infty = \frac{Q_2^2 - Q_1Q_3}{2Q_2 - (Q_1 + Q_3)}
\]

The conditions of mercury concentration in absorbent solution from air were optimized according to the rate of transmission. It was stated that the overshoot is not observed until the rate value is 1 l/min. The limit of mercury detection in air was 10 µg/m³ in a 1 l. volume sample.
A fiber-optic fluorescent immunoassay system of E. coli O157:H7 was developed and applied to a flow immunoassay.

The immunoassay system consists of an optical fiber probe with collective lens, reaction cell with inlet/outlet ports for reagent, and a laser diode for excitation source and a photo diode for detecting fluorescence. The measurement principle is based on sandwich immunoassay. Immune complex with cyanine 5 (Cy5)-labeled antibody was made on the fiber surface by flowing reagents into the reaction cell. An excitation light was transmitted into the optical fiber probe through the lens, and the Cy5-labeled antibody was excited by evanescent wave arose on the fiber. The fluorescence was detected by a photodiode. For detection of a heat-killed E. coli O157:H7 as a target analyte, a goat polyclonal antibody was used as capture or fluorescent-labeled antibody.

As a result of flow measurement, the calibration range for E. coli O157:H7 was from $10^4$ to $10^7$ cells/ml. The measurement time for each sample was within 12 min. The immunoassay system showed high specificity for E. coli O157:H7. To achieve faster immunoassay, changes in fluorescence intensity at binding reaction of Cy5-labeled antibody were evaluated by the binding rate of antibody. The calibration range became narrower ($10^5$ to $10^7$ cells/ml), but the measurement time was shortened to 6 min. Consequently, the flow immunoassay system is suitable for direct and continuous monitoring of microorganisms or bacteria in food, clinical and environmental sources.
Dynamic (non-equilibrium) extraction and fractionation methods involving the continuous provision of fresh extractant volume to the solid sample under investigation have recently drawn much attention as appealing alternatives to the batchwise steady-state counterparts [1-5]. The most important weakness of these flow-based approaches is the low amount of solid that can be handled, typically < 300 mg, whereby they are merely suitable for extraction of trace elements in homogeneous samples; otherwise sample representativeness might not be assured.

To tackle this limitation, we have devised a fully automated flow-through extraction system incorporating a specially designed extraction column with a large volume capacity, wherein up to 2 g of solid sample could be handled. The assembled flow setup was exploited for fast screening of metal pollution in highly inhomogeneous raw municipal solid waste (MSWI) bottom ashes. The pools of readily mobilizable metal forms were ascertained using the toxicity characteristic leaching procedure (TCLP) [6] based on the usage of 0.1 mol/L CH\textsubscript{3}COOH as leachant and analysis of extracts by inductively coupled optical emission spectrometry. A two-level full factorial design was applied to evaluate whether experimental factors such as the solid to liquid ratio, the leachant flow rate and the column disposition (presence or not of fluidized bed conditions) and second order interactions between them are significantly influencing the leachability of trace elements (namely, Cd, Cr, Cu, Pb, and Zn) in MSWI bottom ash. Further details as to the analytical performance of the dynamic extraction method including validation through use of mass balance and the significance of the factors in the multivariate screening test are also presented.
REFERENCES

A multisyringe flow-injection with spectrophotometric detection method is proposed for sulfide ions determination in environmental samples. It is based on the formation and monitorization at 666 nm of the methylene blue through stopped-flow procedure. A 200 cm liquid optical fiber has been used to improve the detection. For method optimization, a central composite design (CCD) was run to optimize the sensitivity (peak area), and a model was build to relate objective functions with experimental conditions.

This system has shown the possibility to combine the determination of another species, as ammonium, in the same manifold.
AUTOMATED SIA SYSTEM FOR SIMULTANEOUS DETERMINATION OF INORGANIC ANIONS IN WATER SAMPLES FOLLOWING VALID ISO NORMS

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SIA can afford easy transfer of flow methods for determination of inorganic anions that are included into valid ISO norms and thus are prepared for routine implementation to analyses carried out by laboratories engaged in environmental monitoring. The automated SIA system for simultaneous determination of selected inorganic anions in water samples was developed. The SIA system fulfills all requirements (mainly calibration ranges) of valid ISO norms describing determinations of individual anions using FIA or CFA systems.

Solutions consumption and analysis time were main parameters evaluated for comparison of simultaneous SIA to FIA (CFA) methods. Small amount of used solutions and generated waste together with shorter analysis time in the compact simultaneous or individual SIA determinations compared to individual anions analyses in the FIA or CFA systems are the proved benefits of the proposed method. SIA was carried out using commercial “one-channel” non-complicated system and there was no need to construct other channels as in the FIA systems.

Determinations of nitrites and nitrates were changed in applied reaction – diazo copulation reaction was used for the determination of nitrites. Nitrates were analyzed by the same reaction after reduction on cadmium column. Chlorides were detected after reaction with mercury(II)thiocyanate, the released thiocyanate ions then reacted with iron(III) in acid solution to form a reddish-brown coloured iron(III)thiocyanate complex which was measured. The analysis of soluble reactive silica was based upon the formation of yellow silicomolybdic acid from the reaction of ammonium molybdate and silica at low pH.

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SEQUENTIAL INJECTION SYSTEM FOR THE SPECTROPHOTOMETRIC DETERMINATION OF AMMONIUM IN PORTUGUESE ESTUARINE WATERS

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Clean unpolluted water is essential not only for human health and well-being but also for sustain earth ecosystems. Plants and animals in lakes, rivers and seas react to changes in their environment caused by changes in chemical water quality. Almost all human activities can and do impact adversely upon the water. Water quality is influenced by both direct point source pollution (from sewage treatment and industrial discharge) and diffuse pollution (from farming) which come from urban and rural populations. For agriculture, the key pollutants include nutrients and pesticides. The European Environment Agency has set a list of potential pollution indicators to monitor coastal and transitional waters, such as ammonium. Large inputs of nitrogen (and phosphorous) to water bodies can lead to eutrophication causing ecological changes that result in loss of plant and animal species, and affect the use of water for human consumption and other purposes. In the form of ammonium, nitrogen is toxic to aquatic life at certain concentrations in relation to water temperature, salinity and pH. Furthermore, ammonium exerts a demand on oxygen in water due to its oxidation to nitrite and nitrate.

Within this context, a flow system for the determination of ammonium in transitional waters was developed. Therefore, the method should cope with a wide range of salinity. Samples were collected in three locations of four Portuguese rivers in the northern Portugal: Ave, Cávado, Douro and Lima. Other samples of interstitial water were also analyzed to evaluate the ammonium accumulation in the river bench.

The determination is based in the colorimetric change of bromothymol blue (BTB) indicator with the presence of ammonia. Ammonium is converted to ammonia by the
mixture with sodium hydroxide; afterwards ammonia diffuses through a gas diffusion unit causing a pH change in the BTB indicator. The pH change of the BTB in the acceptor channel causes the colour change, measured at 620 nm. No BTB solution is consumed as this solution is recirculated back to its container. No interference from different saline levels of the samples was observed.

With the same manifold configuration, two dynamic ranges were achieved just by altering the number of introduced plugs. Some figures of merit of the developed system are summarized in the table below.

<table>
<thead>
<tr>
<th>Dynamic concentration range (mg/L)</th>
<th>Calibration curve</th>
<th>LOD (mg/L)</th>
<th>LOQ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 – 1</td>
<td>(A = 0.156 (\pm 0.003) [\text{NH}_4^+] - 0.082 (\pm 0.016); R^2 = 0.993 \pm 0.002)</td>
<td>0.03</td>
<td>0.09</td>
</tr>
<tr>
<td>1 – 5</td>
<td>(A = 0.0863 (\pm 0.0098) [\text{NH}_4^+] + 0.0775 (\pm 0.0187); R^2 = 0.999 \pm 0.001)</td>
<td>-</td>
<td>0.14</td>
</tr>
</tbody>
</table>

The developed system was applied to the determination of ammonium in estuarine waters with direct introduction of the sample, without any previous treatment. Other samples were also analyzed with the developed method such as water from wells and interstitial water from the bench of the studied rivers.

Acknowledgements:
R.B.R. Mesquita thanks to Fundação para a Ciência e a Tecnologia (FCT, Portugal) the grant SFRH/BPD/41859/2007.
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SHIPBOARD APPLICATION OF AN AUTOMATED MULTIPURPOSE SEQUENTIAL INJECTION ANALYZER FOR IN-LINE WINKLER REACTION

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An analyzer system based on sequential injection analysis technique for the determination of dissolved oxygen in seawater based on the Winkler reaction is presented. Three operation modes were established and successfully applied onboard a research vessel in the Austral ocean:

1. In-line execution of the entire Winkler method including precipitation of manganese(II) hydroxide, fixation of dissolved oxygen, dissolution of the oxidized manganese hydroxide precipitate and generation of iodine and tri-iodide ion with spectrophotometric quantification.

2. Spectrophotometric quantification of iodine in off-line prepared Winkler samples.

3. Titration of iodine with thiosulfate using the syringe as automatic burette.

In the first operation mode, the zone stacking principle was applied by aspiration of three volumes of the sample intercalating small volumes of the both reagents in order to achieve high dispersion of the manganese precipitate in the sample zone. After a short reaction time, the composite plug was dispensed towards the detector with in-line addition of acid in order to dissolve precipitate and generate iodine/tri-iodide. Concentrations and consumption of reagents were highly reduced compared to the classical Winkler protocol and only 2.25 ml of sample was required. Spectrophotometric detection was done at the isobestic wavelength 466 nm of iodine and tri-iodide. The calibration function was linear up to 16 mg l\textsuperscript{-1} following the
equation of $0.025 \cdot \text{AU} \cdot [\text{O}_2]^{-1} + 0.18 \text{ AU}$ during on-board application and an injection frequency of 30 per hour was achieved. The system showed satisfying stability, robustness, and a repeatability of generally < 1.5 % RSD.

In the second mode, the sample was aspirated and pushed through a thermostatization coil into the detection flow cell and quantified at the isobestic wavelength of iodine and the tri-iodide ion. A sample frequency of $90 \text{ h}^{-1}$ was achieved with a mean RSD of 0.8 % ($n = 5$) with an analytical response of $0.043 \text{ l mg}^{-1} \cdot \text{ DO} + 0.009 \text{ AU}$ during on-board application.

In the third mode, a software protocol was established for the dispense of user-defined volumes between 4 ml and 1 µl including the automated refilling of the syringe whenever required, counting the total consumed volume of the titration agent, and, if required, cleaning of the syringe content at the beginning and end of the valuation process. Threefold repetition of titration of seawater samples gave RSD < 0.2 % or differences of < 5 µl titration agent, respectively.

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DIFFERENT APPROACHES FOR THE DETERMINATION OF AMMONIUM IN RAIN WATER BY FLOW ANALYSIS

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In a students project at Hamburg University of Applied Sciences (HUAS) a small river named “Bille” is monitored since 2002. After strong rain events almost all concentrations measured are lower than before the rain fall, because of dilution. Only the ammonium content is usually higher after rain fall.

Therefore different studies have been made to determine ions in rain water. Anions are mostly measured by ion chromatography and ammonium has been measured by different techniques of flow analysis. The results about this topic will be presented here.

Determination of ammonium has been carried out by two different flow injection systems. At one a gas diffusion unit is used and the ammonia transferred is measured by conductivity in a homemade tube in tube electrode according to Tubino [1]. The other flow injection system is based on photometric detection of the Berthelot blue reaction using salicylate instead of phenol.

Rain water samples have been taken at Hamburg-Bergedorf, at Mallorca and at Uberlandia (MG, Brasil). At Brasil the measurements have been done by a simple field photometric procedure [2]. The most detailed investigations at Hamburg have shown that the results of measurements done at the same time at nearby located sampling points differ much higher than expected. This might be due to the sampling procedure, which is not a real wet only sampling. Therefore the dry deposition (which normally fluctuates high even with small changes of sampling location) might be the reason for the distribution obtained.
Fig. 1: Distribution of ammonium values in mg/L obtained at 7 close together located sampling points

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MULTICOMMUTATED FLOW SYSTEM FOR SEQUENTIAL SPECTROPHOTOMETRIC DETERMINATION OF IRON, CHLORIDE AND NITRITE IN WATER

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The quality of drinking water is the subject of increased public attention at the present time. Efficient analytical procedures to determine the analytes which affect water quality, in particular chloride, iron and nitrate, have been cited. Analytical procedures include tests for robustness, low reagent consumption, and reduction in waste generation. The present work reports achievements in developing a multicommuted flow procedure to determine chloride, iron and nitrite in drinking water. Solenoids micro-pump were used to determine the above-cited analytes in sequence. The methods for the determination of iron and nitrite were based on the reaction with 1,10-phenanthroline and Griess reaction, respectively. A reaction with mercury thiocyanate and iron nitrate [1] was used to determine chloride.

The flow manifold system was comprised of seven solenoid micro-pumps used to insert reagents and sample solutions. Under optimum experimental conditions, a linear response ranging from 0.5 to 12.0 mg L\(^{-1}\) Fe (R = 0.9999); 5.0 to 30.0 mg L\(^{-1}\) Cl\(^{-}\) (R = 0.9955) and 0.5 to 4.0 mg L\(^{-1}\) NO\(_2^{-}\) (R = 0.9993) were achieved. Other profitable features such as relative standard deviations of 2.0 % (n = 7), 2.0 % (n = 7) and 3.0 % (n = 7) for typical samples containing 6.0 mg L\(^{-1}\) iron, 15.0 mg L\(^{-1}\) chloride and 2.0 mg L\(^{-1}\) nitrite; detection limits of 0.045; 1.5; 0.014 mg L\(^{-1}\) of iron, chloride and nitrite, respectively; and throughput of 32 determinations per hour were also obtained. The results obtained with the proposed procedure presented agreement with those obtained using reference methods.

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A new method of simultaneous FIA determination of Fe(II) and Fe(III) in water is presented. The method exploits the gradient titration in a system of reversed flow.

In gradient titration, if appropriately large dispersion of the sample zone is achieved, a linear relationship between width of signal registered and logarithm of analyte concentration is observed. Since there is no necessity in such a case to assign neither volume nor concentration of titrant added to a sample, a calibration procedure is necessary to be used. For the aim conventional interpolative calibration method with a set of standards prepared is usually applied.

In the approach developed a small amount of titrant (EDTA) is injected into a stream of sample containing a mixture of indicators (sulfosalicylic acid and o-phenanthroline). As a result of the reaction between Fe(III) and EDTA a peak of characteristic shape is registered. Signal for Fe(II) is measured as the peak height (corresponding to concentration of the Fe(II)-phenanthroline complex) whereas signal for Fe(III) can be measured in the peak width (in accordance to the FI gradient titration rule) or the peak area mode.

The method was verified on example of the determination of Fe(II) and Fe(III) in synthetic samples and then it was applied to analysis of water samples collected from artesian wells in Kraków. Results obtained were compared with those obtained using spectrophotometric batch procedure exploiting o-phenanthroline as indicator. In addition, the total iron was determined in the same samples by ICP-OES technique. It has been revealed that the developed method provides reliable results in terms of precision and accuracy. It is cheap, consuming small amounts of reagents and requiring simple instrumental system. Analysis of a single sample takes several minutes. For these reasons the method can be recommended as a simple tool for evaluation of Fe(II) and Fe(III) content in water samples.
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**DETERMINATION OF ZINC IN NATURAL WATERS USING A MULTISYRINGE FLOW INJECTION ANALYSIS APPROACH COUPLED WITH A LONG LIQUID WAVEGUIDE CAPILLARY FLOW CELL**

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Zinc is a natural microelement important for maintaining the normal physiological processes in living organisms. It is involved in various biochemical processes and is essential for the functioning of enzymes that control protein synthesis and the growth/repair of cells. However, the excess intake is prejudicial and the maximum tolerable daily intake in humans is 1.0 mg kg\(^{-1}\) body weight [1]. For all these reasons it is crucial to develop simple, robust and low cost methods to accurately determine its concentration in water samples and flow based strategies are very suitable for this goal. However, analytical difficulties might arise due to the low concentration level of the analyte.

The spectrophotometric determination can be based on the colorimetric reaction between zinc and zincon. Zincon is a well known spectrophotometric reagent for zinc and copper. It reacts with zinc at pH 8-9.5 and with copper at pH 5-9.5, and at pH 5-7 copper zincon complexes are strongly favoured [2]. Efficient control of pH is therefore crucial to assure selectivity of the method and this is usually achieved by applying buffer solutions with high buffering capacity. However, this approach frequently results in pronounced “schlieren” signal when conventional spectrophotometric flow cells are applied. In the present work, a long liquid waveguide capillary cell with 100 cm of optical path was applied to increase the sensitivity of the spectrophotometric detection mode. For flow manipulation/programming a multi-syringe flow injection analysis (MSFIA) was developed.
The developed flow method offers some advantages as low sample/reagent volumes, low waste volumes with a determination rate of 40 h\(^{-1}\). The developed method is suitable for samples with zinc concentration between 2-80 µg/L. The work is focused on the zinc determination but the development of a multi-parametric system for copper and zinc is the final objective of this work.

Figure 1. Multi-syringe flow injection analysis manifold for the determination of zinc in waters. S\(_i\): syringes; V\(_i\): solenoid valves; SL: sample loop (400 µL); r: reaction coil (200 cm); c\(_i\): confluences; D: detector (100 cm of optical path); CP: computer; W: waste; S: sample or standard; B: buffer; R: color reagent (zincon).

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References:
Sulfate (SO$_4^{2-}$) is the second major anion in water reservoirs. The origin of most sulfate compounds is the oxidation of sulfite ores, the presence of shales, or the industrial wastes. Problems caused by sulfates in natural waters are most often related to their ability to form strong acids which changes the pH. Sulfate ions also are involved in complexing and precipitation reactions which affect solubility of metals and other substances. High sulfate concentrations may interfere in the efficiency of chlorination in water supplies and sulfate salts may increase the corrosive properties of water. High sulfate concentrations in drinking water can impart a bitter taste and also act as a laxative.

The aim of this work is to develop a Multisyringe Flow Injection System (MSFIA) for sulfate determination in drinking and natural waters based in the turbidimetric method. The constructed system consists in the use of a multisyringe burette as an impeller of the sample and reagents (Fig. 1). Measurements were carried out with an Ocean Optics USB2000 UV-VIS Detector. Instrumental control and data acquisition were performed using the software Autoanalysis. The MSFIA burette has been equipped with four syringes with a solenoid valve for each one. The first syringe (S1) dispenses the deionized water carrier. The second syringe (S2) is used for loading and dispensing the magnesium chloride-acetic acid buffer solution. The third syringe (S3) contains the barium chloride solution. The syringe S4 was idle. An additional solenoid valve is needed to manage the sample loading through a coil connected to this extra valve. Two three-way connector and two reaction coil were coupled to the manifold. To carry out
the proposed MSFIA application, sample and buffer solutions are dispensed in order to adjust pH. In the next step, the sample-buffer solution reacts with the barium chloride solution to form a suspension of barium sulfate crystals. The resulting turbidity is proportional to the sulfate concentration of the sample. Preliminary results show that the proposed MSFIA system constitutes an effective approach for sulfate determination. The developed technique offers also typical characteristics of the multicommutated systems, as portability, low reagents consumption and a higher sample frequency.

![Diagram of MSFIA System proposed for sulfate determination.](image-url)

*Fig. 1. MSFIA System proposed for sulfate determination. E1, E2, E3, E4, E5: Solenoid valves; LC: Loading coil; RC1, RC2: Reaction coils; C1, C2: Three-way connectors; D: UV/Vis Detector.*
ON-LINE COUPLING OF MULTIMODAL BEAD-INJECTION INVOLVING REVERSED-PHASE AND MOLECULAR IMPRINTED SORBENTS WITH LIQUID CHROMATOGRAPHY FOR AUTOMATIC SOLID-PHASE EXTRACTION AND DETERMINATION OF TRACE LEVEL CONCENTRATIONS OF CHLOROTRIAZINES IN ENVIRONMENTAL SAMPLES

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Molecular imprinted polymers (MIP) have recently drawn much attention as highly selective solid phase materials for direct isolation of environmental pollutants in raw matrices with no need for further clean-up [1]. Because of the deterioration of the retention capacity for target organic species as compared with reversed phase materials and irreversible sorption of interfering compounds by non-specific interactions the implementation of miniaturized and permanent MIP-based solid-phase (SPE) reactors in flow-systems coupled on-line to column separation systems has not received widespread acceptance. In addition, band broadening effects have been observed whenever MIP eluates are on-line delivered to reversed-phase chromatographic columns.

In this communication a novel method based on bead-injection analysis [2, 3] and involving renewable tandem-SPE columns composed of molecularly imprinted polymers and copolymeric N-vinylpyrrolidone/divinylbenzene beads (Oasis HLB) is proposed for the multiresidue determination of chlorotriazine herbicides (namely, atrazine, simazine, propazine) and principal degradation products (namely, deisopropyl-atrazine and deethyl-atrazine) in raw soil extracts and underground waters at concentration levels below those endorsed by the EU-Water Framework Directive.

The effect of several experimental parameters, such as the dimensions and composition of the SPE microcolumn, the sample loading flow rate, and the eluent volume on the enrichment factors and the disposable nature of the column were investigated in detail.
Particular attention is given to the configuration of the flow setup for appropriate removal of interfering species through use of organic solvent washing, the circumvention of analyte band-broadening by on-line post-SPE dilution and transfer of the eluate into the HPLC via a heart-cut interface.

References

AN IMPROVED CUSTOM-MADE PURGE AND TRAP FLOW SYSTEM USING MICROWAVE ENERGY FOR THE DETERMINATION OF PRIORITY VOLATILE ORGANIC POLLUTANTS IN WATER

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The intensive industrial use of halogenated solvents since the late 19th century has led to serious environmental pollution problems and rendered water supplies unusable for drinking, agricultural, or industrial purposes in many cases. By virtue of their high volatility, these pollutants are scarcely present in surface water, but can frequently be found in aquifers following subsurface percolation. In recent years, the development of methods for determining VOCs has focused on headspace extraction, whether static or dynamic, which provides low detection limits for drinking water but low recoveries from natural waters by effect of the analytes being absorbed by dissolved organic matter.

This communication describes a novel flow extraction method for the determination of the 11 priority volatile organic pollutants in natural water based on fast microwave-assisted extraction followed by sorption of the volatilized analytes on a sorbent column that is subsequently analysed by thermal desorption gas chromatography–mass spectrometry. The extraction efficiency (90–98\%) was maximal for 3 mL of sample (0.75 g of KCl and 5 min extraction time), even for the least volatile compounds (trichlorobenzenes and naphthalene) and in waters containing more than 6 mg/L dissolved organic matter. An anhydrous salt precolumn was included in the flow system in order to reduce water retention on the sorbent column. Quality-related parameters such as linear range (0.1–100 µg/L), limits of detection (0.03–0.1 µg/L) and precision (RSD, 5–8\%) were determined in fortified river water samples. The ensuing
method was successfully applied to the determination of the 11 priority volatile organic pollutants in real water samples.
CONTINUOUS SOLID-PHASE EXTRACTION AND DETERMINATION OF PHARMACEUTICAL PRODUCTS IN WATER SAMPLES BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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The growing use of pharmaceutical products is posing an increasingly serious environmental problem. High concentrations of pharmaceutical products and their metabolites are currently reaching wastewater treatment plants via human urine or faecal excretion, and also as a result of pharmaceutical manufacturing discharges. Their incomplete removal by water purification treatments is resulting in an increasing presence of their residues in waste, surface and drinking waters in a number of countries including Germany, Italy, Greece, Spain, Sweden and USA. Quantitative evaluation of the fate of these chemicals for educated risk assessment and monitoring of drinking water quality requires the use of analytical methods capable of determining concentrations as low as a few nanograms per litre.

Available methods for this purpose involve solid-phase extraction (SPE) followed by gas chromatography–mass spectrometry (GC/MS) or liquid chromatography–tandem MS (LC/MS/MS) detection. The latter has the advantages that it provides detection limits comparable with those of GC/MS and that it is not influenced by the presence of co-extracted substances such as humic acids; however, it has limited resolution and is expensive to implement. GC/MS often requires derivatization, which is usually done with diazomethane, trimethylsulfoniumhydroxyde, N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) or N-methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide (MTBSTFA). Isolation is a critical step in the extraction of pharmaceutical compounds that relies on differences in chemical nature between analytes. Such differences were exploited by our group to develop an SPE–GC/MS method for the analysis of acid,
neutral and alkaline pharmaceutical products. Ten analytes (clofibrin acid, ibuprofen, metoprolol, propanolol, ketoprofen, carbamazepine, diclofenac, estrone, 17β-estradiol and 17α-estradiol) were preconcentrated onto an Oasis HLB minicolumn and eluted with 400 µL of ethyl acetate prior to derivatization with a mixture of BSTFA and trimethylchlorosylane, and injection into a GC/MS instrument operating in the SIM mode. Average recoveries from 100 mL samples were close to 100%, and precision (RSD less than 6 %) and detection limits (< 1 ng L⁻¹) quite good. The proposed method was successfully used to analyse various types of water (pond, river, well, tap, drinking and waste).