Automation and miniaturization of reagent based assays, is a central theme for both research and routine analytical work, since such diverse fields as biochemistry, environmental assays, agricultural assays, biotechnology, oceanography, clinical chemistry, and radiochemistry are critically dependent on this type of assays. Regardless whether the end measurement is carried out by a simple detector (such as spectrophotometer), or a sophisticated one (e.g. MS) the execution of the preceding “wet chemistry” steps, where reagent and sample solution are brought together to react under controlled conditions, is critical to success of any assay. Flow based techniques such as Flow Injection (FI) and Sequential Injection (SI) proved to be well suited for this purpose, as documented in almost 20,000 papers and 20 monographs published on this topic.

The talk will briefly review solution handling concepts, starting with traditional, manual batch techniques to continuous flow methods. The central theme will be the computer controlled programmable flow techniques used by Sequential and Bead injection in miniaturized Lab-on-valve (LOV) format. The principles of BI and SI, will be illustrated by applications from biochemistry and chemical oceanography while focusing on recent development in LOV format – the long optical path flow cell (UV-VIS) and on simultaneous monitoring of absorbance and fluorescence in solution and on beads. In conclusion merits and disadvantages of FI and SI will be briefly summarized and their use for automated solution handling and remote, autonomous monitoring will be outlined.
Recently, efforts have been focused on the miniaturizing of the liquid-liquid extraction procedure by reducing the organic solvent, leading to the development of significant microextraction methodologies like: sequential injection liquid-liquid extraction (SI-LLE), single-drop microextraction (SDME), cloud point extraction (CPE) and wetting film extraction (WFE). A significant advantage of the SI systems is the accurately manipulation of very small volumes of solutions, in the order of some μL, thanks to the use of a syringe pump and the much lower waste production.

Lately, a novel microextraction technique, termed dispersive liquid-liquid microextraction (DLLME) based on ternary component solvent systems, was presented [1]. An appropriate mixture of an extraction solvent (xylene) and a disperser solvent (MeOH), with high miscibility in both aqueous phase and extractant, was injected on-line into the moving sample solution, resulting thus a cloudy solution of fine droplets of extraction solvent. The xylene droplets, which contain the metal complexes, were retained on the PTFE-turnings into the microcolumn. The method has the advantages of high enrichment factor, extreme simplicity, low expense and no need of using conventional segmentor and phase separator units.

The aim of this work was to develop an automatic sequential injection dispersive liquid-liquid microextraction (SI-DLLME) preconcentration system for metal determination coupled to ETAAS. The effectiveness and efficiency of the proposed method has been demonstrated for lead determination in environmental water samples.

This presentation will discuss the benefits gaining by flow based analytical techniques using some natural reagents. Water extract of guava leaf can be used as a reagent for iron determination by employing a simple flow injection (FI) set-up. The extract can be only crude, not necessary to be in pure stage. This takes advantage in that, in a FI system, an analyte could be determined via a calibration under the same conditions as that of standards. The fresh leaves can be taken from a tree in a garden. Green tea leaves (commercially available package for drinking from a convenient store (for tea break in a garden)) can also be used for iron assay by using a simple flow injection (FI) assemble. Using a simple lab-on-chip with time based approach, acidity can be determined by using some natural indicators such as an extract of Butter Fly Pea flowers (taken from a garden) Some others have been exploited. The flow based analysis with natural reagents leads to green analytical chemistry. The analysis can be performed in an open-air in a garden. Discussion on the flow systems and the natural reagents will be made.
Nowadays, miniaturization and automation of measurement devices attract a lot of attention from many researchers dealing with analytical sciences. Automation usually involves fluid dynamics in tubular or microfluidic platform or so-called Lab-on-a-Chip (LoC). Inner diameters (i.d.) of channels of these devices are normally in the range of 0.3 to 1.0 mm or even below 0.3 mm down to 50 μm. Control of liquid flow rate of the devices is strictly important. Therefore, precise and on-line measurement of fluidic flow rate is essential. However, commercial flow meters suitable for these ranges of diameters are rare. Most flow meters available in the market fit with large scale fluidic flows (i.d. ~ 1 cm to 10 m) and are not proper to microfluidic devices.

In this work, development of flow meters will be described. Measurement of flow rate was based on assessing the rate that which air bubble travels within a distance of dual-array detection points. Three kinds of sensor were examined, i.e., light emitting diode (LED), capacitively coupled contactless conductivity detector (C⁴D) and ion-sensitive field effect transistor (ISFET). Comparison will be made amongst adoption of these three types of sensors in term of range of fluid flow and accuracy.
Acknowledgements

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Solid-phase extraction (SPE) has been consolidated as the most versatile sample processing method for removal of interfering species and/or analyte enrichment. Although significant advances have been conducted over the past two decades for automation of the entire analytical protocol involving flow-based SPE, in-line/on-line SPE assays performed in a permanent mode lack sufficient reliability as a consequence of the progressive tighter packing of the bead reactor, contamination of the solid surfaces and potential leakage of functional moieties.

This lecture overviews the current state-of-the-art of bead injection analysis as an appealing analytical tool for overcoming the above well-known shortcomings of SPE when implemented in a flow manifold exploiting a permanent sorptive column [1]. The most relevant asset of bead-injection based procedures is the automated renewal of the sorbent material per assay whereby fresh sorptive surfaces are made available for the analytes in each individual sample. This lecture will address and critically pinpoint novel instrumental developments based on the three generations of flow injection that have been reported for execution of SPE in a bead injection fashion [2-4]. Also described will be the vast number of alternatives proposed in the literature for immobilization of reactants on beads and in-line chemical derivatization as well as the instrumental detection techniques utilised for on-column optosensing measurements or post-extraction detection [3,4]. Relevant environmental and bioanalytical applications reported within the past few years will be also presented and discussed in detail.

References:


Paired emitter-detector diodes (PEDDs) coupled with potentiometric pH-meter or multimeter have been characterized as complete instruments for common photometric measurements as well as optical flow-through detectors. The analytical characteristics of investigated devices have been illustrated by the use of paired LEDs for the determination of model analytes. Measurements with pH-meter resulted in a wide linearity of PEDD response, according to the developed model based on Shockley equation and the Lambert-Beer law. PEDD-based photometric devices generate a potentiometric analytical signal that is directly proportional to the analyte concentration. On the other hand, PEDDs coupled with ordinary voltmeter offer a significant enhancement of sensitivity. Due to high sensitivity, rapid response and high stability as well as durability, low-cost, simplicity of customization and miniaturization, PEDDs are especially attractive as detectors for flow analysis. Several practical applications of developed detectors for the analytical and bioanalytical measurements have been indicated.

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Automatic flow based determination of antioxidant capacity: from total assessment to biological targets

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The formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) has been clearly implicated in the pathogenesis of several human diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders and certain types of cancer [1]. Considering the protective effects of antioxidants against the deleterious oxidative induced reactions involved in these pathologies, interest in antioxidant research has become a topic of increasing attention in the last few years. The existence of simple, convenient, and reliable in vitro analytical methodologies for the fast determination of antioxidant capacity of pure compounds or in complex matrices is essential to this research field. In this context, flow injection techniques are an excellent tool to automate these analyses [2, 3].

In the present communication, the state of the art about automatic flow-based methods for antioxidant assessment will be highlighted. Special emphasis will be given to the specific features of existing flow injection based systems for determination of scavenging capacity against biologically relevant ROS and RNS. Several features will be compared, including the analytical figures of merit, the application to real samples and the “chemistry” behind the assays. Perspectives about novel assays and current lab work will also be presented.
References


A highly selective, interference-free biosensor for the measurement of fructose in real syrup samples was developed. The assay is based on the phosphorylation of D(-) fructose to fructose-6-phosphate by hexokinase and subsequent conversion of fructose-6-phosphate to fructose-1,6-biphosphate by fructose-6-phosphate-kinase. The heat liberated in the second reaction was monitored using an enzyme thermistor. The major advantages of this biosensor are rapid and selective measurement of fructose without the need to eliminate glucose and inexpensive FIA based mediator-free calorimetric measurement suitable for regular fructose analysis. This biosensor was optimized for parameters, such as pH, ionic strength, interference, operational stability and shelf life. The biosensor presented a good linearity (0.5 - 6.0 mM) with a detection limit of 0.12 mM, RSD 1.2% with 99.5% reproducibility. The biosensor was used for fructose determinations in real syrups obtained from a production site. Short- and long-term use of the biosensor over a period of fourteen months gave reproducible results and good overall stability. The method is useful for routine fructose monitoring in food samples.
Simultaneous multicomponent analysis of liquids is an important task. Sensor arrays, multidimensional math method and sequential injection analysis (SIA) are perspective elements of automatic systems for analysis a large number of species in small volume of samples. Potentiometric sensors have some advantages for using as detector, for example, rapid response, direct dependence from ion concentration etc. but each sensor has individual electrical contact and so quantity of the sensor in the array has limitation in few elements on cm$^2$ and it impose difficulties to miniaturized of the detection unit. In this case Light Addressable Potentiometric Sensor (LAPS) is perspective tool because light for actuation has down to 1 micron in diameter. Thus it is possible place on the 1 cm$^2$ few decades of the sensors. This area allow construct miniature flow cell for analysis of the sample volume less then 50 μl which is sufficient for concentration measurements in biological and medical samples.

The designed automatic system is shown schematically in Figure. For pump solutions and deliver them to the flow cell a syringe pump (1) is used, while a multiposition valve (2) permits to select among various solutions (calibration solution or samples). A flow cell (17 μl inner volume) with incorporated the sensor array chip was developed and made from polymethylmethacrylat (PMMA) plate with the help of the micromilling technique. Different milled parts of the PMMA were glued together by methacrylic acid. LAPS chip with 120 sensitive membranes with 0,25 mm in diameter was fixed with flow cell by PDMS (Polydimethylsiloxane). Laser positioning was carried out in two directions by stepper with 0,1 micron precision of the steps. Before analytical measurements optimization of the parameters of the system were carried out. Dependences of the analytical signal (current amplitude) from light modulating frequency, light amplitude,
DC voltage shifting, etc. were investigated. As example, three types of the photocured polymer sensitive membranes with well known characteristics (sodium, potassium, chloride) were deposit on LAPS and model mixed solution were analysing in $10^{-5}$-$10^{-1}$ mol/l concentration range. Analytical characteristics and possible applications of the proposed system will be demonstrated in the presentation.
A simple, rapid and sensitive spectrophotometric method for the determination of Paracetamol using merging zone-continuous flow injection analysis via hexagonal flow cell and homemade photometric based on 704 nm LED and a photo silicon mini detector was studied.

The method was based on the oxidation of Paracetamol by Fe(III) solution which is lead to formation of Fe(II) which in turn, reacts with potassium hexacyanoferrate to form the Prussian blue dye and determination of this dye at 704 nm.

Chemical and physical parameters were investigated. The analytical graph is ranged from 0.0 – 10 mM L\(^{-1}\) with a limit of detection of 2.0 nM.L\(^{-1}\) and use of analysis of variance (ANOVA) for the treatment of data. The relative standard deviation was %0.08 for 6.0 mM.L\(^{-1}\) Paracetamol solution (n=8) with sampling rate was 60 sample/h. The method was applied for the determination of Paracetamol in pharmaceutical formulations.
THE MARRIAGE OF FLOW ANALYSIS TECHNIQUES AND AQUATIC BIOGEOCHEMICAL STUDIES: SOMETHING OLD, SOMETHING NEW, SOMETHING BORROWED......

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This paper describes the development of new methods and applications of flow analysis for the study of nutrient biogeochemistry in aquatic ecosystems. One of the earliest gas diffusion methods described in flow injection was for the determination of carbon dioxide. New variations on this approach are now being applied in preference to conventional methods, for the determination of total alkalinity and carbon dioxide in the study of anthropogenic CO₂-induced acidification of the oceans. The rate of CO₂ production is also a commonly used measure of the mineralization of organic matter in marine and estuarine sediments, but conventional sampling methods can disturb the sediments and suffer from poor spatial resolution. A new gas-diffusion sampling and analysis probe is described that is capable of fine scale profiling of CO₂ in unconsolidated sediments.

Further developments in the mapping of total nitrogen (TN) and total phosphorus (TP) in marine and estuarine systems using flow systems incorporating on-line digestion, are also described. The on-line determination of TP has been validated, and shown to work reliably for an extended period. However, the conditions required to achieve rapid and efficient mineralization of organic N in a flow system, generate a serious interference in the detection of the nitrate, which is used as the basis for quantification of TN. Some old approaches to this new problem have been applied with encouraging results.
REAL-TIME SIMPLEX OPTIMIZATION OF BATCH-WISE AUTOMATION OF SILICATE IN SEAWATER EXPLOITING MULTISYRINGE FLOW INJECTION AND MICROPUMP TECHNIQUE

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A new flow analyzer (Fig.) based on batch-wise and parallel automation is presented. The system enable the fast transfer of sample using a micropump and addition of required reagents from two selection valves by syringe pumps to four independent, thermostated, and atmospherically open mixing chambers on will according a software instruction protocol. After defined reaction times, the chambers are emptied by aspiration through the detection flow cell for quantification of the reaction products. Cleaning protocols for the chambers and reagent tubes have been established. The presented assembly enables the automation of nearly all spectrophotometric methods.

Here, we present an analytical method for silicate in seawater samples based on the molybden blue methodology and carried out on the presented analyzer assembly. Real-time optimization applying the modified SIMPLEX procedure of the composition of the first reagent containing sulfuric acid and ammonium molybdate (prepared on-line from stock solutions) and three reaction times (t1: before addition of oxalate, t2: before addition of ascorbic acid, and t3: before detection) was performed.

Two surrogate seawaters was measured in duplicate, one containing 10 µM of phosphate as blank standard and one containing 20 µM silicate as standard aiming a maximization of the weighted response function: 

\[ ((\text{Abs}(SO_4) - A \cdot \text{Abs}(PO_4))/B)^{(t1+t2+t3)} \]

with A and B being user defined coefficients.
Real-time SIMPLEX optimization and the corresponding software protocol as well as the analytical performance, and application of the proposed analyzer are discussed thoroughly in poster format.
A flow injection (FI) method with chemiluminescence (CL) detection for the determination of picomolar concentrations of total dissolved cobalt (dCo) in open ocean waters is presented. Cobalt is determined via its enhancement of the oxidation of pyrogallol by hydrogen peroxide in a basic medium in the presence of the surfactant, cetylammonium bromide (CTAB). Seawater samples are typically acidified to pH 1.7 but this treatment did not completely dissociate the cobalt from organic ligands naturally present in seawater. Therefore dCo was underestimated and it was necessary to include a UV-irradiation step prior to FI-CL detection.

The effect of UV-irradiation was demonstrated on a Pacific Ocean sample from the SAFe (Sampling and Analysis of Fe) intercomparison programme. A concentration of 40.9 ± 2.6 pM dCo ($n = 9$) in UV-irradiated sub-samples was determined, compared with 21.3 ± 0.6 pM dCo ($n = 8$) in non-irradiated sub-samples. These results were in excellent agreement with other international laboratories and confirms the importance of the UV-irradiation step for quantitative recovery of dCo. Results are also presented for UV-irradiated, vertical cast samples from the Sargasso Sea, an oceanic region in which carbon fixing phytoplankton have an absolute requirement for cobalt.

This FI-CL method has the capability to provide high temporal and spatial resolution dCo data. Given the direct link between dCo and ocean productivity in certain open ocean regimes, the method is a useful tool for investigating aspects of climate change and the global carbon cycle.
AUTOMATIC ASSESSMENT OF THE EFFECT OF THE DIFFERENT PHENOL DERIVATES ON THE CHEMILUMINESCENCE LUMINOL-H2O2-HORSERADISH PEROXIDASE SYSTEM EXPLOITING MULTISYRINGE FLOW INJECTION ANALYSIS

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The luminol-H₂O₂-horseradish peroxidase (HRP) system has been employed to quantify a wide variety of analytes who act as enhancers or inhibitors. Numerous derivates of phenol act as substrates, donating hydrogen to the enzyme HRP and depending on its structure they could exhibit an enhancing or inhibiting effect of this chemiluminescence (CL) system. The investigation of such effect would clearly benefit from a rapid and precise fluidic manipulations as well as reaction conditions by automated flow-through methods which are not yet available. Among the currently available flow analysis techniques, Multisyringe Flow Injection Analysis (MSFIA) has been consolidated as a robust flow methodology which offers versatility of flow management and multichannel operation in a wide flow rates. In addition, CL detection offers great analytical advantages over the other techniques as it is selective, sensitive, simple, inexpensive and rapid.

The aim of the present work is the development of a MSFIA set-up for the assessment of the effect of different phenol derivates on the luminol-H₂O₂-HRP system. For this purpose, it is performed an in-line reaction of H₂O₂, phenol derivate and HRP prior to the fast reaction with the luminol, profiting from the features of this methodology. Several studies were performed with the aim of establishing the appropriate flow system configuration and reactions conditions. Current work is focused in the classification of
the different phenolic derivatives in relation with their CL response, in order to assess the potential applicability of the proposed system for the determination of groups of phenols or their total amount in different analytical fields, such as pharmaceutical, food and/or environmental analysis.

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ON-LINE ANALYSIS OF VFA’S IN ANAEROBIC TREATMENT PROCESSES.

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In anaerobic treatment processes (in sewage sludge treatment plants, food industries, industrial production of ethanol ...), Volatile Fatty Acid (VFA) concentration is usually the process control parameter, and is often determined by off-line procedures (sampling followed by an extraction step before analyses), inducing thus a delay for analytical results. Moreover, these procedures often involve chromatographic or potentiometric separation leading to non-direct, time-consuming and material-demanding analyses that are not prone to fieldwork applications. Within the most reported methods are included ion-exclusion, liquid and gas-chromatography with sample pre-treatment as distillation or headspace chromatography or with a direct injection. In fermentation processes and anaerobic treatment of wastewaters or solid wastes, end-product (ethanol, methane ...) or intermediate products (VFA) inhibition occurs, which results in reduced process efficiency and stability; therefore control measures should be taken in continuous operation fermenters. End-product inhibition control cannot be achieved properly if on-line or on-site measurements of the product concentration are not performed. In this context, some researchers have proposed adapted system for on-site analysis based on titration method: the “5-point Titration Method” has been developed by Moosbrugger and co-workers, and an improvement of this method has then been proposed by Lahav et al., involving eight pH observations. These titration methods suffer from many interferences, such as presence of metallic ions which can form complexes with carboxylic groups, and are found to be either too elaborate or too approximate for general practical application.

In this work, a MSFIA method was developed for a simple, rapid and accurate measurement of volatile fatty acids (VFA) in various environmental samples. This fluorimetric method involves a derivatization step of SCFA with N-(1-naphthyl)ethylenediamine (EDAN), and allows on-line determination of the sum of
acetic, butyric, propionic and valeric. The derivatization and direct fluorimetric detection conditions of total small aliphatic carboxylic acids were optimized, according to experimental plan depicted in Figure 1 (with an example of its application for acetic acid).

Figure 1. Derivatization and detection pattern for acetic acid.

1st step: activation of carbon bearing -OH group with EDC

2nd step: Fluorescent labelling with EDAN

Key parameters of derivatization (pH, reaction time) and detection (pH, reaction time) steps were optimized by a step by step approach. Detection of the fluorescent amide products being obviously hindered by the simultaneous presence of the excess fluorescent amine reagent (EDAN), an on-line separation method of these two fluorescent compounds was integrated into MSFIA system.

Validation of the on-line system developed was assessed by application of the procedure to aqueous samples originating from sewage sludges. The results were in good agreement with analysis by HPIC.
SIMULTANEOUS DETERMINATION OF CYANIDE AND THIOCYANATE BY GAS DIFFUSION-FLOW SPECTROPHOTOMETRY BASED ON THE FORMATION OF TETRACYANONICKELATE(II)

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The instantaneous reaction of cyanide with nickel(II) in ammoniacal buffer to form tetracyanonickelate(II) which characteristically absorbs UV light at 267 nm was successfully used as a basis for simultaneous determination of cyanide and thiocyanate using gas diffusion-flow spectrophotometry. The cyanide, which is directly converted into molecular hydrogen cyanide in acid donor stream, then, diffuses through the membrane in the gas diffusion cell, and it reacts with nickel(II) in ammoniacal buffer acceptor stream to form tetracyanonickelate(II) which is detected spectrophotometrically at 267 nm. Whereas, thiocyanate has to be previously oxidized by Cerium(IV) in the acid donor stream to form cyanide, which is then reacted with nickel(II) in ammoniacal to form tetracyanonickelate(II). Thus, simultaneous determination of cyanide and thiocyanate was done firstly, by introducing sample into acid donor stream to obtain cyanide only, and secondly, by introducing of sample into acid donor stream containing Cerium(IV) to obtain the total cyanide and thiocyanate. Thiocyanate was obtained by subtracting the first run with the second run.

The proposed method has been validated and applied for simultaneous determination of cyanide and thiocyanate samples from urine and photographic wastewaters with satisfactory results.
A laboratory-assembled automated pretreatment system (Auto-Pret System) was successfully designed in this work. The system was equipped with home-written operating program prepared by using Visual Basic programming software. The chitosan-based chelating resin, packed in a mini-column and utilized as an on-line preconcentration device, was installed to the Auto-Pret System coupled with spectroscopic detection. The system offered fully automated, easy operation and powerful analytical tool for separation, collection/concentration and determination of trace- and ultratrace- elements in environmental samples in a short time and accompanied with excellent detection limit. In comparison to the commercially available flow-based system, the Auto-Pret System is more robust, versatile, less reagent consumption and less waste production since the continuous flow system can be avoided. More importantly, the chitosan-based chelating resins developed in combination with the automated pretreatment system, satisfied the requirement for the improvement of selectivity, sensitivity, precision, accuracy, rapidity, and reproducibility in analytical chemistry.

The developed chitosan-based chelating resins in this work provide better characteristics than those of commercially available, especially in term of adsorption kinetics, adsorption capacity, selectivity, as well as separation of matrix interferences. Some trace elements determined in the samples are Cr (VI), Cr (III), Pb, Cd, Ag, Be, Co, Cu, Ni, U, V, Mo, Hg, and rare earth elements (REEs).
A new flow injection chemiluminescence system developed for determination of anions like sulphate. The method is based on displacement of equivalent amount of bromide ion on an Br⁻ from ion-exchange minicolumn by sulphate. The released Br⁻ is then reacts with BrO₃⁻ in acidic medium to produce bromine water which catalyzes the Luminol/H₂O₂ chemiluminescence. Working conditions for these determinations were optimized. The emission intensities produced were recorded as peak height (mm) on a chart recorder and they were proportional to the amount of sulphate injected. This method allowed determination of 0.96 mg/l SO₄²⁻.

The developed FIA-CL system was applied for determination of sulphate in water (well and waster water) in different regions in Erbil City – North of IRAQ. Interfering effects of various anions were eliminated by inserting a suppressor minicolumn in the FIA-CL system after injection port, which allowed for sulphate only to pass through. The method provided very simple, rapid and sensitive method for sulphate determination without any pretreatment.

References:
Two simple methods were developed for the estimation of thiocyanate ions in human saliva, in the presence and absence of SDS-surfactant. A comparison was performed in terms of precision, accuracy, sample throughput, cost and reliability. The methods can be used as a comparison mean to distinguish smokers from non-smokers persons. Different physical and chemical parameters were optimized. In these two methods, thiocyanate ion was injected into acetic acid carrier solution which reacts later with hypochlorite stream to form cyanogen chloride and this react with nicotinamide stream before coupling with barbituric acid. Thus, the pink color formed is monitored during the stopped period in the flow cell. The calibration graph was constructed in the absence of SDS by employing reaction time of 4.5min. Nicotinamide in the concentration range of 0.5-30.0µg/ml (R= 0.9987 and D.L= 0.37µg/ml) was determined. In the SDS micellar medium an increasing in the reaction product stability and sensitivity of the method was observed. Thus, linear relation in this case was obtained at 2min reaction intervals and concentration range of 0.08-16.00 µg/ml (R= 0.9991 and D.L. 0.03µg/ml). The results obtained from both methods are compared with those obtained by spectrophotometric method.
COMPUTER-CONTROLLED FLOW-BASED CHEMICAL ANALYSIS SYSTEMS ASSEMBLED WITH PLUNGER-TYPE PUMPING MODULES: THEIR ADVANTAGEOUS AND DISADVANTAGEOUS FUNCTIONS

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Computer-controlled flow-based analysis systems were assembled using plunger-type pumping modules, 12-port, 8-port or 6-port selection and switching valve modules, solenoid valves and pumps; they are a flow-injection and a sequential-injection system, a mini-column pretreatment system (Auto-Pret system), an Auto-Pret/HPLC system, an Auto-Pret/FIA system and a SIEMA (Simultaneous Injection Effective Mixing Analysis system). Such systems are fully controlled by a programmed computer: the programs used is lab-made with Visual Basic.

In this work, analytical results obtained for typical analysis methods are demonstrated and the usefulness and advantages of the plunger-type pumping modules are discussed.

One of the most interesting advantages is a flow stream under “high pressure” of plunger-type pumping devices, which is very useful for a column pretreatment method and a high-pressure liquid chromatographic method. Other advantages are: long-life and stable pumping efficiency, low-cost maintenance and many kinds of devices. The most inconvenient point of the plunger-type pumping device is: the total volume of pumping is fixed for each device, and therefore changing the total volume of pumping needs changing the pumping device.
USE OF GLYCOGEN PHOSPHORYLASE b AS A RECOGNITION ELEMENT FOR AN EFFECTOR IN COMBINATION WITH A FIA TECHNIQUE

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Glycogen phosphorylase (GPb) immobilized onto porous glass beads with controlled pore size (CPG) was packed into a small polymer column and then, assembled into a photometric flow injection system. The system was applied to measure variation in the catalytic activity of the enzyme column as follows:

\[(\text{Glycogen})_{n-1} + \text{Glucose 1-phosphate} \rightarrow (\text{glycogen})_n + \text{orthophosphate}\]

Changes in the enzymatic activity were measured through monitoring the amounts of orthophosphate liberated in the enzyme-catalyzed reaction. Microdetermination of orthophosphate was based on the malachite green method modified. Injecting a substrate solution containing 5.0 % glycogen and 0.25 mM glucose 1-phosphate (G 1-P) into the system almost did not increase absorbance at 650 nm in the effluents from the column. However, introduction of the substrate mixed with 1.0 mM adenosine monophosphate (AMP) as an effector molecule for the GPb into the system showed enhancement in absorbance at 650 nm, indicating orthophosphate enzymatically formed. Increases in absorbance at 650 nm with increase in AMP with various concentrations were observed.

Thus, the photometric flow injection system for determination of the effector molecules in combination with the corresponding allosteric enzyme could be exemplified.
Sequential injection analysis (SIA) provides lower reagent consumption and waste generation than conventional flow injection analysis (FIA). In an SIA format, mutual penetration of sample and reagent(s) zones is essential for a successful chemical reaction. However, it is usually difficult for SIA to obtain a well-mixed condition of these zones. This lower mixing efficiency plagues the utilization of SIA in some cases.

2-(5-Bromo-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl)amino]aniline (5-Br-PSAA) reacts with palladium to form a complex which has an absorption maximum at 612 nm. This chemistry is introduced into a newly proposed simultaneous injection-effective mixing analysis (SIEMA) system which can accomplish effective mixing of solution zones. The SIEMA system shown in Fig. 1 basically consists of a bidirectional pump (P), three 3-way solenoid valves (3V) and a 2-way solenoid valve (2V) which are controlled by homemade software in a laptop computer. Palladium standard/sample, 5-Br-PSAA and acetate buffer (200 μL each) are sequentially aspirated into each holding line (HL) via each 3-way solenoid valve. During aspirating them, the 2-way valve is turned off for selective aspiration with accurate volume via an interested 3-way solenoid valve (to avoid aspiration of fluid via other two 3-way solenoid valves). Then all
solutions are simultaneously dispensed to be merged at the confluence point (4C2). After the confluence point, an effective mixing condition can be obtained and therefore the complex formation takes place successfully in the mixing coil. The colored product is measured with spectrophotometric detector.

The 3σ limit of detection was 2.5 µg L⁻¹ palladium, and the linear range extended to 4.0 mg L⁻¹ ($r^2 = 0.999$). The SIEMA system operated on automated 82 sec cycle for one determination. The reagent consumption was $6.0 \times 10^{-8}$ mol 5-Br-PSAA (200 µL of $3.0 \times 10^{-4}$ M 5-Br-PSAA) for one determination. This is less than that in FIA procedure (approximately $1.6 \times 10^{-7}$ mol) [1], thus the proposed SIEMA could save reagent consumption.


Fig. 1 Flow diagram of the SIEMA system for Pd determination. C, water; P, syringe pump; AC, auxiliary coil (2 mm i.d., 0.65 m long); 4C1, 4-way cross connector; HL, holding line; 3V, 3-way solenoid valve; S, standard/sample; R, $3 \times 10^{-4}$ M 5-Br-PSAA; B, 0.2 M acetate buffer (pH 4.5); MC, mixing coil (0.8 mm i.d., 1.2 m long); 2V, 2-way solenoid valve, D, spectrophotometer (612 nm); R, recoder; PC, computer; W, waste.
LOW PRESSURE ION CHROMATOGRAPHY IN OPEN TUBULAR COLUMNS: A SIMPLE TOOL FOR MULTIANALYTE DETERMINATIONS OF IONIC SPECIES.

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Flow injection analysis (FIA) offers a simple, fast, sensitive and inexpensive way of analyzing various samples. Due to the complexity of most of the analyzed mixtures however, a pre-separation step is often required if more than one compound is determined. Such a pre-separation step that is typically incorporated in the flow system is not very selective, so in order to achieve high selectivity for each analyte in a multianalyte mixture, complex manifolds with a multitude of flow channels and detectors are required. This considerably complicates the otherwise very simple and straightforward flow analysis schemes. Combination of FIA with various separation methods such as electrophoresis and chromatography is possible (FIA-CE, FIA-LC), and the analytical power of these combined methods is impressive. However the cost of such a system is a sum of the cost of the flow analysis and separation system and can be quite considerable, especially when liquid or ion chromatography is coupled to FIA. It would be therefore desirable to incorporate a simple, chromatographic step into a FIA manifold without a need to modify the typical FIA setup. We present here an approach that is conceptually midway between micro flow analysis and separation methods, notably ion chromatography. We have recently developed a concept of low pressure ion chromatographic separations in open tubular capillary columns [1-3]. The columns are typically 50 to 75 μm ID fused silica capillaries, 1-5 m long. They are coated with a multi-layered stationary phase that takes care of the separation of the ions from the samples, thus providing the required selectivity. The stationary phase is attached to the wall of the capillary and does not increase the pressure required to pump the eluent (carrier) solution through such a column. Typical backpressure in such a system is less than 20 kPa and a simple instrumentation such as low pressure peristaltic pump can be applied. In its simplest version, the pumps can be removed and the flow of liquids can
be achieved by gravity by placing a liquid reservoir to a defined height above the system. A simple and inexpensive contactless conductometric detector is used for sensitive detection of separated ionic species. Although the separation power, expressed in terms of separation efficiency, is not as impressive as in a typical IC system based on packed columns, the open tubular approach presents an instrumentally simple and cheap alternative. In this contribution we will discuss some basic concepts in constructing a low pressure IC system, discuss in detail the preparation of capillary columns based on various polymerization schemes and finally we will present some application of low pressure IC in multianalyte determination of inorganic ions.

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DEVELOPMENT OF SEQUENTIAL INJECTION CHROMATOGRAPHY FOR MORE ROBUST AND PRECISE METHOD

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Sequential injection chromatography (SIC) has been considered over years as an alternative to HPLC technique for analysis of simpler (less complex) samples. Although the manifold of commercial SIA was tuned up year by year, implementation of chromatographic column together with increased inner pressure generates until now hidden weak points. Thanks to higher working inner pressure limits of particular parts of system are exceeded. Then the system is not suitable to long term trouble-less measurement with satisfactory results similar to HPLC.

Tuning was focused on all parts to ensure optimal flow path with low dispersion – same I.D. of all parts, no micro leaking, stable selection valve, proper detector setup with narrow bore flow cell and pulse-less pump. All parts of SIC manifold should be developed with regard to typical HPLC manifold.

Applications of new sizes of monolithic columns in SIC present new trend in comparison with particle columns from which SIC still can not profit.

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THE POTENTIAL OF MULTISYRINGE CHROMATOGRAPHY FOR FAST, SENSITIVE AND SELECTIVE DETERMINATION OF TRACE PHARMACEUTICALS IN COMPLEX MATRICES

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For the one hand, the most powerful methodologies for the determination of pharmaceuticals and personal care products are usually based on liquid or gas chromatography coupled to mass spectrometry. Initially these compounds were determined in pharmaceutical formulations and biological fluids, but the interest their determination at low levels in environmental and waste water samples is currently growing up.

For the other hand, Flow Analysis Techniques have demonstrated to be a powerful tool in the automation and miniaturization of analytical methodologies. Among them, the Multisyringe Flow Injection Analysis Technique (MSFIA) has been consolidated as a versatile and robust flow technique, which allows analytical operations at relatively high pressures. Bearing in mind that highly porous monolithic columns provide good separation efficiencies at lower pressures than classic particulate columns, the development of the so-called Multisyringe Chromatography (MSC) technique was achieved.

The aim of this work is the development of a fast, sensitive and selective MSC system for the determination of trace pharmaceuticals in complex samples, in order to achieve a simple alternative prior the use of more complex instrumental techniques. This is accomplished exploiting in-line disk-based solid phase extraction for the enrichment and matrix isolation of the target compounds prior to their chromatographic separation (C18
monolithic column), and followed by post column reaction with tris(2,2’-bipyridyl)ruthenium(III) (generated on-line from tris(2,2’-bipyridyl)ruthenium(II) and Ce(IV)) and chemiluminescent detection. In this first application, the proposed instrumental set-up has been applied to the determination of thiazide compounds commonly used as pharmaceuticals, which could be potentially found in wastewater samples.
Solid-phase extraction (SPE) is a common tool for sample preparation. Despite their attractive features, as ease of automation and the wide range of sorbents commercially available, the non-selective interactions present in the classical sorbents is a frequent source of impaired performance caused by the partial co-extraction of interfering substances. In this context, the use of solid-phase extraction based on molecularly-imprinted polymers (MIP) could easily enhance the performance of the analytical methods by performing the molecular recognition of the target analytes, allowing a selective extraction. This fact is particularly important when complex matrices are handled.

In the present work an automatic method for the selective extraction and on-line determination of riboflavin in food samples is proposed. This new approach was based on the use of a commercially available MIP for riboflavin as sorbent, a multisyringe-lab-on-valve analysis (MSFIA-LOV) as automation tool and a conventional liquid chromatograph as separative technique. By using MSFIA-LOV system, it was possible to handle µL of bead suspension for sorbent renewal, and also to adjust the eluate composition, avoiding band broadening effect during the chromatographic analysis. Different strategies for handling the bead suspension and for performing the elution were evaluated. The method was successfully applied to the quantification of riboflavin in...
complex food samples as infant milk, liver digests and energetic drinks without any prior treatment.

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Guanosine derivatives are important for diagnostics of oxidative DNA damage. 8-hydroxy-2′-deoxyguanosine (8-OHdG) is one of the most abundant products of DNA oxidation. Its determination in biological fluids, e.g., urine is possible. Nevertheless, due to its low concentration and matrix interferences (uric acid) using HPLC without previous extraction is difficult.

In this study, we tested the isolation of 8-OHdG from biological matrix by using paramagnetic particles with antibody-modified surface. The recoveries in different matrices and analyte concentration were estimated. After the isolation, the analyte was determined by flow injection analysis with tandem UV and electrochemical detector. The UV detection was carried out at 260 nm. For electrochemical detection, we used an amperometric cell with planar glassy carbon electrode. After the optimization of the measuring parameters, we obtained a linear concentration dependence within the range from 1 to 1,000 µM with a reliability coefficient $R^2=0.998$. Detection limit was estimated as 10 nM.
A study of biospecific interactions between lectins and glycoproteins based on quartz microbalance technique has been carried out. Measurements were performed in a flow-through mode using panel of four different lectins and commercially available QCM-D instrument with dissipation monitoring. Plant lectins were covalently immobilized on a surface of gold QCM chip through the standard amino-coupling technique. The working conditions of the assay (flow rate, lectin concentration, regeneration conditions) were optimized. Frequency shift was used as an analytical signal for qualitative and quantitative analysis of the glycoprotein adsorption to the various lectin surfaces. Dissipation shift provide additional information about viscoelastic properties of the formed layer. A calibration between 0.15 and 3.0 μM for fibrinogen and 0.1 mg/mL and 1.0 mg/mL for asialofetuin with good linearity were obtained. Efficient regeneration procedure for the lectin surface made it possible to reuse surfaces and thus to reduce the summary cost of the assay. Application of regeneration solution such as glycine-HCl (pH 2.5) caused complete removal of glycoproteins from the lectin surface without damaging it. For more than 200 samples R.S.D. of less than 10% was achieved. The lectin-based quartz crystal microbalance biosensor described here enables a convenient, economical flow injection procedure for rapid screening and selective and specific assay of serum glycoproteins. The possibilities for further development and application of the technique for monitoring and control of serum protein glycosylation processes look very promising. It is also a promising alternative to other label-free techniques, such as surface plasmon resonance.
FLOW INJECTION CHEMILUMINESCENCE METHOD FOR THE DETERMINATION OF ACETYLSALICYLIC ACID WITH LUMINOL-HYDROGEN PEROXIDE-POTASSIUM HEXACYANOFERRATE SYSTEM

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A simple and sensitive flow injection chemiluminescence (FI-CL) system proposed for the determination of acetylsalicylic acid (aspirin) in pharmaceutical formulations. The method is based on inhibition effect of aspirin on the chemiluminescence reaction of luminol – hydrogen peroxide and potassium hexacyanoferrate as catalyst. The different experiment parameters affecting the chemiluminescence intensity were carefully studied and incorporated into the procedure. The detection limit was (0.5 μg/ml) aspirin and CL emission intensity was correlated with the drug concentration in the range (2.5-125 μg/ml) with correlation coefficient of (0.9979) and a RSD of < 5(n=5). The results obtained for the assay of pharmaceutical preparations compared well with those obtained by the official method in British Pharmacopoeia and demonstrated good accuracy and precision.
Among all the flavonoids, which display a significant array of pharmacological activity, quercetin (2-(3,4-dixhydroxyphenyl)-3,5,7-trixhydroxy-4H-1-benzopyran-4-one) is most commonly presented in food such as onions, tea, apples and red wine\textsuperscript{[1,2]}. Quercetin is important dietary constituent because it is most widespread and consequently, the most studied flavonoid. A lot of articles are dealing with beneficial pharmacological properties of quercetin in the field of allergy, vascular, inflammation, virology and carcinogenesis\textsuperscript{[3-6]}. As all flavonoids, quercetin exhibits potent antioxidant activity, due to free radical scavenging\textsuperscript{[7]}.

Simplified analytical technique is required for the quercetin determination in food and pharmaceutical dosage forms.

Various methods have been described for the determination of quercetin mostly from plant materials: HPLC\textsuperscript{[8-10]}, capillary electrophoresis\textsuperscript{[11]} and derivative spectrophotometry\textsuperscript{[12]}. Quercetin was also determinated spectrophotometrically via coloring complexing reaction with many different inorganic reagents added\textsuperscript{[13,14]}.

In the present investigation a new method for determination of quercetin in some natural products (cherry, onion, and tea) were developed by reversed FIA-CL method. The method was based on the inhibition of CL emission in the KMnO\textsubscript{4}/luminol/H\textsubscript{2}O\textsubscript{2} system by addition of quercetin.
The reverse flow was used to avoid continuous monitors of CL which leads to unstable baseline. Various parameters associated with this flow system were studied and essential optimizations were carried out. The linear range of the method for determination of quercetin was \((8.0 - 190 \ \mu g/ml)\) with correlation coefficient of \((0.9914)\) and a sampling frequency 80 samples/h. Possible interferences were studied and the results showed that the interferences caused less than 5% error. The method was applied successfully for the determination of quercetin in various natural products.

**References:**

A NEW FLOW INJECTION SPECTROFLOURIMETRIC METHOD FOR THE DETERMINATION OF HOMOCYSTEINE

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Homocysteine is an important amino acid which contains a free thiol moiety and therefore, it has several important roles in physiological matrices [1]. The determination of homocysteine as an important biomarker for a wide range of diseases has gained high interest in the biomedical community over recent years [2].

In this work, a new simple and sensitive flow injection method is developed for the determination of homocysteine with spectrofluorimetric detection technique. This method is based on the oxidation of homocysteine with TI (III) in acidic media, producing fluorescence reagent, TlCl3 2− (λex=237nm, λem=419nm). The effects of chemical parameters (including pH of the solutions, the buffer, potassium chloride and Ti (III) concentrations), instrumental parameters (such as flow rate of the solutions, reaction coil length, sample loop volume) and temperature on the fluorescence intensity as an analytical signal are studied and optimized. In the optimum conditions of the above variables, homocysteine can be determined in the range 0.40 to 40 µM with the LDR from 0.40 to 16.0 µM. The detection limit (with S/N=3) is 0.06 µM of Homocysteine and precision for the injection of 5.0, 10.0 and 15.0µM of Homocysteine are 0.8%, 1.5% and 2.5% (n=10) respectively. The rate of analysis is 90 samples per hour.

The influence of potential interfering substances, including amino acids and carbohydrates is also studied. The proposed method has been successfully used for the determination of homocysteine in the real sample (blood serum and tap water) matrix.

References:
Automation of liquid–liquid extraction procedure based on a novel dual-valve (DV-SIA) approach has been carried out. The main idea behind this new design was to construct a universal SIA system by connecting two independent units, one for aqueous-organic mixture flow and the second for organic phase flow. As a result, the DV-SIA manifold consisted of an Extraction unit, a Separation unit and a Detection unit.

Mixing both solvents leading to extraction step could be provided in two ways - programming flow reversals within the Extraction unit or by air bubbling in the Separation unit. Separation of both phases is not carried out at continuous flow, as is currently done by most of on-line solvent extraction systems, but “manual like” in a miniaturized Separation unit.

The efficiency of DV-SIA extraction was demonstrated by the separation of picric acid in the form of an ion associate, with subsequent spectrophotometric detection. Reaction conditions for ion-associate formation, such as acidity, concentration of dye, organic solvent and detection wavelength, were optimised, as were the physical parameters for extraction in the sequential injection system.

The aim of the presented work was to build universal and robust system easily controllable by lab staff for routine analyses without the need to fully understand principles of sequential injection.

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In the present work, a solid phase extraction (SPE) is hyphenated with MSFIA-system, in order to improve the selenium (IV) determination. Selenium (IV) reacts with aromatic o-diamines such as 2,3-diaminonaphthalene (DAN). The complex formed in the reaction of selenium (IV) with DAN is 4,5-benzopiazselenol. The reaction is greatly influenced by acid concentration, temperature, the length of time employed for color development, and the presence of foreign ions.

On standard procedure for selenium determination a liquid-liquid extraction with cyclohexane is required to piazoselenol extraction. In our system the piazoselenol is retained onto a membrane disk. Two kind of membrane disk (C-18 and poly(styrenedivinylbenzene) copolymer) were investigated. Different organic solvents were tested to piazoselenol elution.

The piazoselenol formed in the reaction of selenium with DAN can be detected by spectrophotometry or fluorometry. Both techniques were compared in our system.
SPE-MSFIA system. Ms (Multisyringe), SV (solenoid valve), T (thermostatic bath; T1=80°C T2=20°C), M (Membrane), D (Detector), W (Waste).
NEW METHOD FOR SPECTROPHOTOMETRIC DETERMINATION OF TOTAL ARSENIC, ARSENIC (V) AND ARSENIC (III) BY FLOW-INJECTION ANALYSIS – MERGING OF REACTION ZONES FOR THE RELEASE OF IODINE FOR THE SYSTEM (As (V) + I⁻ + H₃O⁺)

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A new flow-injection spectrophotometric analysis method has been established for the determination of total Arsenic, Arsenic (V) and Arsenic (III) in the aqueous solutions. This method is based on the reaction of As(V) with iodide ion in acidic media.

Iodine has a maximum of absorption at 350 nm. A graph of absorbance versus concentration shows that the Beer's Law is obeyed over the concentration range of 0.002-230 ppm when merging 25 ml of the sample and 35 ml of iodide and using an acid as a carrier.

Both sample and iodide were injected through a homemade fife-ways plastic value. Physical properties (flow-rate, sample volume, dispersion, reaction coil length and temperature), and chemical properties (iodide concentration, acid concentration) were studied. The limit of detection for n=10 was 0.002 ppm. Sample throughput was 50 samples per hour.

The new method was successfully applied to determine total As, As(V) and As(III). This method is rapid, accurate, sensitive, selective and inexpensive.
A simple, rapid, reproducible and sensitive spectrophotometric method for determination of Cobalt (Co\(^{2+}\)) was investigated. The method was based on the interaction of Co\(^{2+}\) with 1-(2-pyridylazo)2-naphthol (PAN), in the presence of buffer pH 6 (HCH\(_3\)COO + NH\(_4\)CH\(_3\)COO) to give a highly colored species of a molar ratio 1:2 (Co:PAN). Beers law was obeyed in the range of (0.1-2.5) μg/ml with the molar absorptivity of 3.77x10\(^4\) L.mol.cm\(^{-1}\) at \(\lambda\) max 525 nm. The method was adapted to semi automated flow injection system. The molar absorptivity was 0.16x10\(^4\) L.mol.cm\(^{-1}\) at \(\lambda\) max 525 nm. Beers law was obeyed in the range (0.3-10) μg/ml. The precision and accuracy studied to both systems. The method was applied successfully to the assay of Co\(^{2+}\) in real sample such as real water and vitamins, and was well agreed with its certified value.
FLOW METHODS FOR DYNAMIC SYSTEM MONITORING: “OLD” APPROACHES TO “NEW” CHALLENGES

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Over the years flow injection analysis and other automated flow techniques have been acknowledged as powerful analytical tools for serial wet chemical assays and for the study of kinetics of chemical interactions. Nowadays, its scope is broadening into environmental research and into biotechnology as a tool for the study of biological processes. In these dynamic processes, accurate and fast assessment of chemical parameters is essential.

In this work, some applications of the different flow strategies to dynamic problems are discussed and versatility of the applications is demonstrated through various examples as, i) a flow injection method for monitoring cell membrane damage of wine lactic acid bacteria and in Listeria innocua; ii) a sequential injection flow assay for monitoring glycerol in a sugar fermentation process by Saccharomyces cerevisiae; iii) a sequential injection lab-on-valve system for the on-line monitoring of hydrogen peroxide in lens care solutions. Special emphasis is given to the challenging difficulties inherent to the dynamic systems under study.

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A flow injection protocol to assess the potential release of dissolved reactive phosphorus (DRP) by enzymatic hydrolysis of dissolved organic phosphorus (DOP) in natural waters under environmentally relevant conditions is presented. This protocol enables the quantification of different classes of DOP compounds using a variety of phosphatase enzymes i.e. alkaline phosphatase, phosphodiesterase and phytase. All experiments were carried out within the pH range of most natural waters i.e. at neutral (pH 7) or slightly alkaline pH (pH 9). Tri-sodium citrate and sodium dodecyl sulphate (SDS) were used in the assays to prevent interferences due to adsorption processes in the presence of multivalent metallic cations and to minimize protein binding.

This protocol was used to study the speciation of DOP in the temperate Tamar estuary of south west England. The results showed that the DOP pool in the water column varied temporally and spatially within the estuary (1.1 - 22 µg P L⁻¹) and constituted 6 - 40 % of the total dissolved phosphorus (TDP) pool. The enzymatically hydrolysable fraction (EHP) of the DOP pool ranged from 1.1 – 15 µg P L⁻¹ and represented a significant and potentially bioavailable phosphorus fraction. The EHP fraction is not commonly determined in aquatic systems due to the lack of a suitable measurement technique and these results have important implications for phosphorus biogeochemistry, estuarine ecology and the development of efficient strategies for limiting the effects of phosphorus on water quality.
Pervaporation flow injection (PFI) has been successfully applied in combination with hydride generation and photometric detection to the direct determination of arsenite (As(III)) in ‘dirty’ industrial and environmental samples. The use of on-line hydride generation results in the successful separation of As(III) from the sample matrix thus substantially reducing matrix interferences. However, the detection technique requires a suitable colour reaction for the detection of arsine in the acceptor stream. An alternative detection approach involving direct amperometric measurement of arsine in the acceptor stream was proposed and implemented in a simple and inexpensive PFI system. A method for the speciation of As(III) and As(V) was also developed. The proposed PFI system was validated using hydride generation atomic absorption spectrometry. There was no statistically significant difference at the 95% confidence level between the results for the two methods. The PFI method is characterised by a wide analytical concentration range (0.05 to 60 mg L\(^{-1}\)), a detection limit for As of 1.0 µg L\(^{-1}\) and a sample throughput of 12 h\(^{-1}\).
A RAPID AND SIMPLE FLOW-INJECTION ANALYSIS OF LACTOSE USING IMMOBILISED CELLOBIOSE DEHYDROGENASE AND SCREEN-PRINTED ELECTRODES

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The rapid and simple detection of lactose that makes it possible to carry out the high-throughput screening of milk products is the great interest of the dairy industry. Also, the development of a highly sensitive method for lactose control, especially in lactose reduced or lactose free products is important because millions of adults worldwide suffer from lactose intolerance. The conventional techniques for the detection of lactose are expensive and require the skilled personnel. Also time and labour consuming sample preparation steps might be needed in order to carry out the accurate analysis. The enzymatic assay of lactose usually involves the application of two enzymes that makes the analysis more complex and sometimes decreases its selectivity. We propose simple, rapid and sensitive flow analysis of lactose using the amperometric mono-enzymatic biosensor.

The analytical device consisted of cellobiose dehydrogenises (CDHs) from white-rot fungi \textit{T.villosa} and \textit{P.sordida} immobilised onto the working part of the screen-printed carbon electrode served as a transducer. CDHs obtained from white rot fungi are only able to convert celldextrins and lactose but strictly discriminate against other oligosaccharides and monosaccharides. That eliminates the possible contribution of the other sugars present especially in “lactose-free” milk (glucose) and makes the analysis highly selective. The assay was carried out in the flow-injection mode. The working conditions of the lactose detection – flow rate, potential, pH of the carrier buffer - were found and optimised. A flow rate of 0.57 mL min\textsuperscript{-1} provided a good combination of both high and rapid analytical response for the chosen system. Low working potential 100 mV was chosen in order to carry out the detection with high sensitivity and at the same time to minimise the interference from the components present in the sample. The
determinations were performed in 0.02 M citrate buffer with 0.1 M KCl at pH 4.5. The analytical properties of the biosensor based on unmodified carbon electrodes (SPCE) and the ones modified with multi-walled carbon nanotubes (SPCE-MWCNT) were examined and compared. SPCE-MWCNT showed better analytical response than SPCE. That might be explained, perhaps, due to the enhanced surface area of the electrode providing the adsorption of the larger amounts of the enzyme. The linear ranges of the analytical devices were 1-200 μM and 1-100 μM for the sensors based on CDHs from *T.villosa* and *P.sordida*, respectively. The LOD was 250 nM for both of them. The biosensor based on CDH from *P.sordida* showed higher analytical signal, and was chosen for the further experiments. The used biosensor kept its working characteristics more than a week while stored in the buffer. Also the transducer is re-usable, the enzyme can be immobilised on the working surface of the same screen-printed electrode up to 3 times without deterioration of the working properties of the electrode.

The cross-linker such as glutaraldehyde (GA) or poly(ethylene glycole) diglycidyl ether (PEGDGE) were used for the modification of the screen-printed electrodes. It was observed that the cross-linking improved the working properties of the biosensor, i.e. enhanced its analytical response significantly (up to 10 times) and increased the stability. The immobilized enzyme kept 100% of its initial activity even after 8 hours (more than sequential 120 injections) while the biocatalytic response of the biosensor without cross-linker decreased by 15-17% during few hours.

The developed biosensor was tested for the detection of lactose in milk with different percentage of fat, lactose-free milk and yoghurt. It was shown that no sample pretreatment except simple dilution 1:10000 (1:100 for lactose-free milk) is needed. The sensor was stable in milk with 3% of at 1:1000 dilution. No decrease of the response was observed after more than 120 injections of lactose during few hours. The results obtained using CDH-based biosensor were in a good correlation with the data claimed on the packages of the dairy products. The assay was highly throughput, making possible to analyze 50 samples per hour. The proposed assay of lactose is simple, sensitive and reproducible (RSD of 0.3-1.1%). The developed biosensor should be a good alternative as a miniaturised analytical device for the rapid, reliable and inexpensive on-line control, monitoring and detection of lactose in food analysis.
A novel combined thermometric and amperometric biosensor for lactose determination based on immobilised cellobiose dehydrogenase.

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A novel method for lactose determination in milk was proposed. It was based on oxidation of lactose by cellobiose dehydrogenase (CDH) enzyme from the basidiomycete Phanerochaete chrysosporium, immobilized in the enzyme column. The column was prepared by cross-linking the CDH on a controlled pore glass (CPG) beads using glutaraldehyde. Detection was performed in FIA mode by combination of two techniques. First, thermometric response from the enzymatic reaction was measured with enzyme thermistor (ET). Outlet from ET was connected with the electrochemical cell where amperometric signal from reduction of mediator (1,4-benzoquinone, presented in a buffer solution) on graphite electrode was measured with three-electrode potentiostat. No enzyme was immobilized on a graphite surface. Linear response for lactose between 0.25 mM and 20 mM was obtained with the high degree of reproducibility. For more than 500 samples R.S.D. of less than 10% was achieved. The assay time was 3.2 min. per sample. The developed sensor was applied for determination of lactose in dairy milk samples (milk with 1.5% and 3% of fat content and lactose free milk). No sample preparation except dilution was needed for 1.5% and 3% milk samples. However, lactose free milk samples were centrifuged and filtrated prior assay. The proposed method is rapid, suitable for repeated use and allow to achieve correlated results from two different detection methods.
A flow-based strategy to implement differential kinetic analysis is proposed and applied to spectrophotometric catalytic determination of iron and vanadium in Fe-V alloys. To this end, three successive plugs of the sample are rapidly inserted into the same carrier stream, and the resulting overlap between them leads the formation of a sample zone presenting regions associated to local maximum and minimum concentrations. Measurements related to these regions tend to be more precise, and were used for building-up and calibrating the multi-parametric models. The goal of this strategy is the possibility of determining two or more analytes which reacts with the same reagent(s) at different rates as it minimizes the errors due to time synchronism system.

The method relies on the influence of Fe(II) and V(IV) on the rate of the iodide oxidation by Cr(VI) under acidic conditions [1]; therefore the Jones reductor is needed. The sample is inserted into an acidic KI solution that acts also as carrier stream, and a Cr(VI) solution is added by confluence. Measurements related to the maximal and minimal values of the absorbance vs time function were selected, each one being related to a different yet reproducible condition for reaction development. Data were treated by multivariate calibration involving the PLS algorithm [2].

The proposed system is very simple and rugged. Two latent variables are enough carry ca 94% of the analytical information, and the results are in agreement with inductively coupled argon plasma – optical emission spectrometry.

Laboratory flow analysis is being developed for needs of various branches of chemical analysis more than half a century, and has its well recognized place in modern analysis. This is proved by vast number of research papers and developed methods, increasing number of commercially available instruments and increasing number of methods, which are recognized by national and international authorities as standard methods.

Commonly, as major advantages of flow methods of analysis are considered possibility of efficient on-line methods of sample processing prior to the detection step, improvement of precision and efficiency compared to batch procedures, and in some special cases enhancement of limit of detection regardless sample dispersion in flow conditions. In case of widely used the measurements of transient signals in flow injection methods, still rather rarely are exploited kinetic effects. The only effects which are commonly employed are biocatalytic reactions for determination of substrates or inhibitors and trace determinations of several inorganic analytes based on their catalytic effect on some redox reactions.

Kinetic discrimination can be achieved in flow measurements by exploiting differences in kinetics of reactions in solution or kinetics of heterogeneous reaction in detector, and also kinetics of selected operation of sample processing in flow conditions. Utilization of such processes may lead to improvement of selectivity of determination of given analyte
compared to determination in non-flow conditions. In particular cases kinetic effects may be used for multicomponent determination without need of design complex manifolds, use of multi-detector systems or employing chromatographic or electromigration separation methods. Based on literature review and own research numerous such flow-injection systems with optical and electrochemical detections will be presented for single analyte determinations, and also some unique examples of multi-component determinations.