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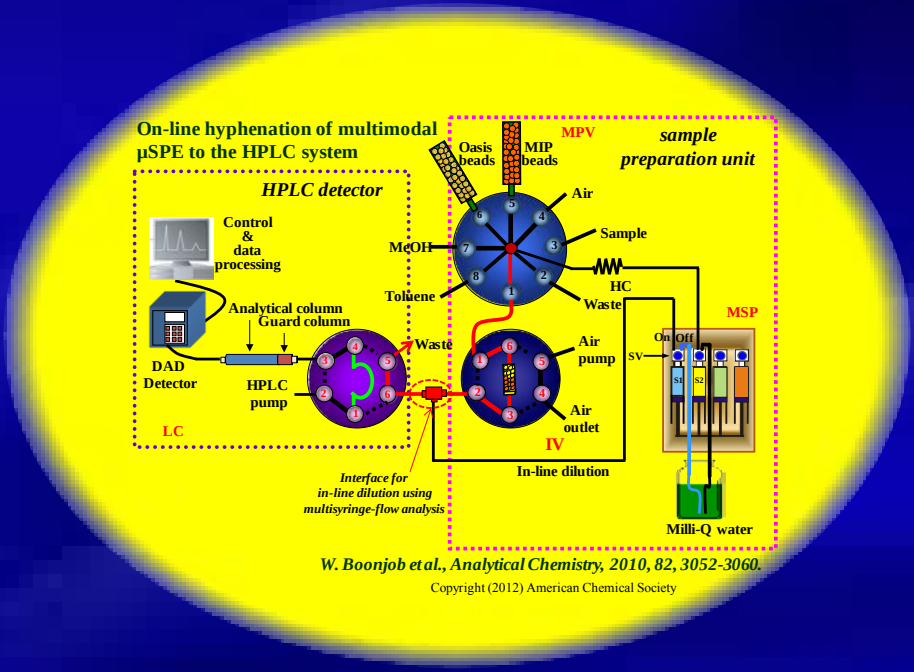
Automated sample preparation methods for the determination of trace level concentrations of environmental pollutants



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July 2012

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Department of Chemistry, Analytical Chemistry Area

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CERTIFY THAT:

Miss Warunya Boonjob with X9237823-B has written herself the Ph.D. dissertation entitled "*Automated sample preparation methods for the determination of trace level concentrations of environmental pollutants"* which has been developed at the Department of Chemistry, Analytical Chemistry Area, University of the Balearic Islands, Spain, as a requirement to receive the Doctor of Philosophy (Ph.D.) in Science and Chemical Technology with International Mention.

Palma de Mallorca, 14 May 2012

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PREFACE

This thesis represents a part of the requirements for obtaining the International Ph.D. degree in Science and Chemical Technology at the Department of Chemistry of the University of the Balearic Islands (UIB). The study was financed with a Ph.D. stipend granted by the Conselleria d'Educació, Cultura i Universitats, Direcció General d´Universitats, Recerca i Transferència del Coneixement from the Government of the Balearic Islands through European Social Fund (ESF) during the time frame 2008-2012.

The papers presented in this thesis have been conducted in different Universities, *namely*: (i) Department of Chemistry at UIB, Spain, (ii) Physical-Chemistry Department, University of Porto, Portugal, (iii) Institute of Food Analysis and Research, Department of Analytical Chemistry, University of Santiago de Compostela, Spain, and (iv) Process Chemical Centre Laboratory of Analytical Chemistry, Åbo Akademi University, Finland.

This thesis is based on the following 7 publications in peer-reviewed journals, which are integrated parts of the thesis, describing the development of automatic sample preparation methodologies for extraction, fractionation, preconcentration, separation and determination of trace levels concentration of organic and inorganic priority pollutants in environmental samples exploiting flow-through based analytical approaches.

i. *Multiple Stirred-Flow Chamber Assembly for Simultaneous Automatic Fractionation of Trace Elements in Fly Ash Samples Using a Multisyringe-Based Flow System*, Warunya Boonjob, Manuel Miró, and Víctor Cerdà, Analytical Chemistry, 2008, **80**, 7319-7326.

ii. *Critical Evaluation of Novel Dynamic Flow-Through Methods for Automatic Sequential BCR Extraction of Trace Metals in Fly Ash*, Warunya Boonjob, Maria Rosende, Manuel Miró, Víctor Cerdà, Analytical and Bioanalytical Chemistry, 2009, **394**, 337-349.

iii. *On-line Hyphenation of Multimodal Microsolid Phase Extraction Involving Renewable Molecularly Imprinted and Reversed-Phase Sorbents to Liquid Chromatography for Automatic Multiresidue Assays*, Warunya Boonjob, Yongliang

Yu, Manuel Miró, Marcela A. Segundo, Jianhua Wang and Víctor Cerdà, Analytical Chemistry, 2009, **82**, 3052-3060.

iv. *Flow-through Dispersed Carbon Nanofiber-Based Microsolid-Phase Extraction Coupled to Liquid Chromatography for Automatic Determination of Trace Levels of Priority Environmental Pollutants*, Warunya Boonjob, Manuel Miró, Marcela A. Segundo, Víctor Cerdà, Analytical Chemistry, 2011, **83**, 5237-5244.

v. *Automatic Dynamic Chemical Fractionation Method with Detection by Plasma Spectrometry for Advanced Characterization of Solid Biofuels*, Warunya Boonjob, Maria Zevenhoven, Paul Ek, Mikko Hupa, Ari Ivaska, Manuel Miró, Journal of Analytical Atomic Spectrometry, 2012, **27**, 841-849.

vi. *On-Line Coupling of Bead Injection-Lab On Valve Analysis to Gas Chromatography (BI-LOV-GC): Application to the Determination of Trace Levels of PolyChlorinated Biphenyls (PCBs) in Solid Waste Leachate Samples*, Jose Benito Quintana, Warunya Boonjob, Manuel Miró, and Víctor Cerdà, Analytical Chemistry, 2009, **81**, 4822-4830.

vii. *Elucidation of associations of ash-forming matter in woody biomass residues*, Warunya Boonjob, Maria Zevenhoven, Paul Ek, Mikko Hupa, Ari Ivaska, Manuel Miró, Fuel, 2012 (Submitted).

ABSTRACT

Automatic sample preparation methods are nowadays imperative to meeting compressed analytical timeline. As a result, mechanized sample preparation methods hyphenated with analytical techniques exploiting the different generations of flow analysis were in this dissertation developed and characterized for determination of organic and inorganic pollutants in environmentally relevant samples and industrial wastes. The thesis consists of two parts; the first is devoted to the development of automatic methods for dynamic chemical fractionation and investigation of bioaccessibility of inorganic trace contaminants in solid samples including coal fly ash and biomass fuels. The second part involved the development of automatic sorptive methods prior to chromatographic assays for extraction, preconcentration, separation and determination of trace concentration levels of selected pesticides (*namely*, triazine and metabolites thereof, and polychlorinated biphenyl compounds (PCBs)) in environmental samples at levels below those endorsed in current Directives.

Dynamic flow-through fractionation is proven to afford more accurate evaluation of potentially bioaccessible metal pools under environmentally changing conditions than the equilibrium-based counterparts as a consequence of the solid/liquid equilibria shift and absence of metal redistribution effects. In fact, natural processes are occurring under dynamic rather than static conditions as assumed in classical methods. In this context, a novel miniaturized flow-based configuration capitalized on stirred-flow cell extraction was devised for automatic assessment of bioaccessible pools of trace metals (*namely*, Cu, Cd, Ni, Pb and Zn) in three samples at a time with no limitation of sample amount up to 1.0 g. A two-step sequential extraction scheme involving water and acetic acid (or acetic acid/acetate buffer) was utilized for reliable estimation of readily mobilisable fractions of trace elements in fly ash under worst-case conditions following the US-Toxicity Characteristic Leaching Test (TCLP), the results of which are reported in chapter 3 entitled "*Multiple stirred-flow chamber assembly for simultaneous automatic fractionation of trace element in fly ash samples using a multisyringe-based flow system"*. In dynamic extraction approaches, the solid sample under investigation is loaded into a suitable container, and exposed continuously to fresh extractant volumes by resorting to flow-based approaches. In this thesis, two dynamic extraction systems, the so-called sequential injection microcolumn extraction (SI-MCE) and sequential injection stirred-flow chamber extraction (SI-SFCE) were critically compared on the basis of the sample-containing container, sample representativeness, homogeneity of the sample, and analytical results aimed at further harmonization of this novel leaching methodology.

The three-step EU approved BCR sequential extraction scheme was performed in both automatic dynamic fractionation systems to evaluate the extractability of Cr, Cu, Ni, Pb and Zn in a standard reference material of coal fly ash (NIST 1633b) as detailed in chapter 4 entitled "*Critical evaluation of novel dynamic flow-through methods for automatic sequential BCR extraction of trace metals in fly ash"*. On-line coupling of SI-SFCE with ICP-OES was resorted to the exploration of the potential availability of ash-forming elements (e.g., K, Ca, Na and Mg) of biomass fuels (*namely*, bark and twigs) in flue gases, which is regarded as indicative of potential fireside problems (fouling and slagging in combustion devices). Experimental results are compiled in chapter 5 entitled "*Automated dynamic chemical fractionation method with detection by plasma spectrometry for advanced characterization of solid biofuels"*. The ultimate aim is to have a reliable system at hand to assist in deciding on a short notice whether or not firing biomass fuels on the basis of potential corrosion risks in combustion devices. For elucidation of metal-biomass/ash associations and investigation of the actual selectivity of extractants, dynamic extraction data was judiciously combined with spectroscopic characterization techniques, *namely*, scanning electron microscopy with energy dispersive X-ray fluorescence spectrometry (SEM-EDX) and X-ray diffraction (RXD) assays. These studies are shown in chapter 6 entitled "*Elucidation of associations of ash-forming matter in woody biomass residues"*.

In the second part of the thesis, automatic sample preparation methods have been developed using solid-phase extraction (SPE) approaches in flowing stream systems as a "front end" to chromatography techniques (GC or LC). Selective μSPE in a Bead-Injection (automatic renewable SPE) mode in the so-called multi-dimensional SPE combining molecular imprinted polymers (MIP) and reversed-phase sorbents (Oasis HLB) was utilized in Chapter 7 (*Multimodal bead injection-based flow-through microextraction involving renewable molecularly imprinted and reversed-phase sorbents as a front end to liquid chromatography for automatic multiresidue assays)* for selective preconcentration of triazine residues and their metabolites in crude soil extracts. The hyphenated μSPE-HPLC system was proven to provide sufficient sensitivity and reliability for determination of the target herbicides (*namely*, atrazine, simazine and propazine) and their dealkylated metabolites (*namely*, deisopropyltriazine (DIA) and deethylatrazine (DEA)) at concentration levels below those specified by current legislations for human water consumption and surface waters. In chapter 8 entitled "*Flow-through dispersed carbon nanofiber-based microsolid-phase extraction coupled to liquid chromatography for automatic determination of trace levels of priority environmental*

pollutants" dispersed carbon nanomaterials were handled as sorptive surfaces in an automatic flow-mode with minimum nanoparticle agglomeration and negligible pressure drop. The proof-of-concept of the method was demonstrated for μSPE and clean-up of chlorotriazine residues (*namely*, atrazine, simazine and propazine) and their dealkylated metabolites (*namely*, deisopropyltriazine (DIA) and deethylatrazine (DEA)) in both environmental waters and soil extracts. In Chapter 9 entitled "*On-line coupling of bead injection-lab on valve analysis to gas chromatography (BI-LOV-GC). Application to the determination of trace levels of polychlorinated biphenyls (PCBs) in solid waste leachates"* is for the first time demonstrated the coupling of mesofluidic platforms with renewable μSPE to GC for monitoring of organic pollutants. This new approach fostered the isolation, concentration, separation and determination of PCBs from raw landfill leachates.

RESUMEN

La automatización de métodos de preparación de muestra es un tema de gran actualidad para reducir tiempos de análisis y mejorar las propiedades analíticas de ensayos químicos. Como consecuencia, en esta tesis doctoral se proponen nuevos métodos de preparación de muestra automáticos y acoplados a técnicas analíticas usando las diferentes generaciones de análisis en flujo para la determinación de contaminantes inorgánicos y orgánicos en muestras de interés ambiental y en residuos industriales.

La tesis doctoral se compone de dos partes: La primera se centra en el desarrollo de nuevos métodos automáticos de fraccionamiento químico dinámico e investigación de la bioaccesibilidad de trazas de contaminantes inorgánicos en muestras sólidas, como por ejemplo, cenizas volantes y biomasa vegetal. La segunda parte incluye nuevos métodos automáticos de (ad/ab)sorción previos a ensayos cromatográficos para la extracción, preconcentración, separación y determinación de niveles traza de varias familias de pesticidas (ej., triazinas y sus metabolitos, y compuestos bifenilos policlorados (PCBs)) en muestras ambientales a concentraciones inferiores a las legisladas.

A diferencia de los métodos de fraccionamiento basados en equilibrio, los métodos dinámicos en flujo son capaces de cuantificar con mayor exactitud la fracción de metales potencialmente bioaccesible al cambiar las condiciones ambientales como consecuencia del desplazamiento de los equilibrios sólido/líquido y la inexistencia de efectos de readsorción de metales. De hecho, los procesos naturales ocurren en condiciones dinámicas en vez de estáticas, que son asumidas en los métodos clásicos. En este contexto, se diseñó un sistema en flujo miniaturizado basado en la extracción en un reactor agitado para la cuantificación de forma automática de la fracción bioaccesible de elementos traza (Cu, Cd, Ni, Pb y Zn) en tres muestras simultáneamente y con cantidades de muestra variable hasta 1.0 g. Se utilizó un método de extracción secuencial de dos etapas basado en el uso de agua y ácido acético (o tampón ácido acético/acetato) como extractantes para la determinación de las máximas fracciones móviles de elementos traza en cenizas volantes mediante el procedimiento americano de lixiviación característica de toxicidad (US-TCLP). Los resultados experimentales se detallan en el capítulo 3 titulado "*Fraccionamiento automático y simultáneo de elementos traza en cenizas volantes usando*

un sistema múltiple de reactores agitados". En las extracciones dinámicas la muestra a analizar se coloca en un recipiente adecuado y se expone continuamente a nuevos volúmenes de extractante mediantes sistemas en flujo. En esta tesis, se comparan dos sistemas de extracciones dinámicas basados en el uso de microcolumnas o reactores agitados en sistemas en flujo (Inyección secuencial, SIA) en cuanto al tipo de recipiente para la muestra, representatividad y homogeneidad de la muestra y resultados analíticos, con el objetivo final de armonizar esta nueva metodología de lixiviación. Para ello se evaluó la extractabilidad de Cu, Ni, Pb and Zn en un material de referencia certificado de cenizas de carbón (NIST 1633b) utilizando el método BCR de tres etapas recomendado por la UE. Este trabajo se incluye en el capítulo 4 titulado: "*Evaluación crítica de nuevos métodos dinámicos en flujo para la extracción automática de metales traza en cenizas usando el método BCR de extracción secuencial*". Para investigar la posible disponibilidad de elementos responsables de la generación de cenizas (ej., K, Ca, Na y Mg) en biomasa (corteza y ramas de árboles) en gases de combustión se diseñó un sistema en-línea acoplando extracción dinámica basada en reactores agitados a un espectrómetro de plasma acoplado inductivamente con detección óptica. Las fracciones móviles de metales alcalinos y alcalino-térreos son indicadoras de posibles problemas en los sistemas de combustión (formación de depósitos y pérdida de eficacia del reactor) dónde se utilice la biomasa como combustible. Los resultados experimentales se describen en el capítulo 5 titulado: "*Método de fraccionamiento químico dinámico con detección elemental por espectrometría de plasma para la caracterización avanzada de biocombustibles sólidos*". El objetivo final de este trabajo es disponer de un método analítico fiable para decidir a la mayor brevedad posible si diferentes tipos de biomasa podrían ser utilizados como combustibles en base a los riesgos de corrosión en reactores. Para elucidar asociaciones entre ceniza/biomasa y metales e investigar la selectividad real de los extractantes, se combinaron los resultados de extracción dinámica con técnicas de caracterización espectroscópica, como por ejemplo, la microscopía electrónica de barrido con espectrometría de fluorescencia de rayos X dispersiva (SEM-EDX) y difracción de rayos X (RXD). Estos estudios se incluyen en el capítulo 6 titulado: "*Elucidación de asociaciones de elementos responsables de la generación de cenizas en residuos de biomasa*".

En la segunda parte de la tesis se incluyen nuevos métodos automáticos de preparación de muestra basados en la extracción en fase sólida (SPE) en sistemas en flujo acoplados a técnicas cromatográficas (GC o LC) para la determinación de contaminantes orgánicos. En el capítulo 7 titulado: "*Microextracción en fase sólida basada en bead-injection multimodal*

usando polímeros de impresión molecular y materiales de fase reversa para análisis automático de multi-residuos de herbicidas/pesticidas" se propone un método µSPE selectivo con fase sólida renovable de forma automática, denominado *Bead-Injection-SPE* multi-dimensional, combinando polímeros de impresión molecular (MIP) y materiales de fase reversa (Oasis HLB) para la preconcentración selectiva de residuos de triazinas y sus metabolitos en extractos crudos de suelos. Se demostró que el acoplamiento µSPE-LC proporciona suficiente sensibilidad y fiabilidad para la determinación de los analitos a niveles de concentración inferiores a los legislados en aguas de consumo humano y aguas superficiales. En el capítulo 8 titulado: "*Método de extracción en fase sólida miniaturizado basado en la dispersión de nanofibras de carbono en sistemas en flujo para la determinación automática de niveles traza de contaminantes orgánicos prioritarios mediante LC*" se utilizaron nanomateriales de carbono como adsorbentes en técnicas en flujo sin problemas de aglomeración ni aumento de la sobrepresión en el sistema automático. Para demostrar el potencial analítico de este método se analizaron trazas de herbicidas de la familia de las clorotriazinas (atrazina, simazina y propazina) y sus metabolitos (desisopropiltriazina (DIA) y desetilatrazina (DIA) en aguas y extractos de suelos previa preconcentración por µSPE. En el capítulo 9 titulado: "Acoplamiento en-línea de bead-injection-Lab-on-Valve a cromatografía de gases *(BI-LOV-GC). Aplicación a la determinación de niveles traza de bifenilos policlorados (PCBs) en lixiviados de residuos sólidos"* se propone por primera vez en la bibliografía el acoplamiento de plataformas mesofluídicas con µSPE renovable a GC para la monitorización de contaminantes orgánicos. Mediante este nuevo método se determinaron PCBs en lixiviados crudos de residuos en vertederos previa extracción y concentración automática seguida de separación por GC.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
A	surface area
AA	ascorbic acid
AAS	atomic absorption spectroscopy
ACE	automated cartridge exchange
AEDT	additional expenditure due to treatment
BCR	the commission of the european communities bureau
BI	bead injection
BI-SPE	bead injection solid phase extraction
CAR	carboxen
CC	central communication conduit
CL	central communication line
CMS	chromatographic mode sequencing
CNF	carbon nanofibers
CNTs	carbon nanotubes
C_0	the initial concentration of a given analyte in the sample
CW	carbowax
D	detector
DCB	dithionite-citrate system buffer with $NaHCO_3$
DDD	dichlorodiphenyldichloroethane
DDE	dichlorodiphenyldichloroethylene
DDT	dichlordiphenyltrichloroethane
DLLME	dispersive liquid-liquid micro-extraction
DVB	divinylbenzene
EP TOX	extraction procedure toxicity
FAAS	flame atomic absorption spectroscopy
FC	flow cell
FIA	flow injection analysis
GF	gas filtration
HC	holding coil
HCH	hexachlorocyclohexanes

LIST OF ABBREVIATIONS (CONT.)

HF-LPME	Hollow fiber-liquid phase microextraction
HLB	hydrophilic-lipophilic-balanced
HPD	high-pressure dispenser
HPLC	high-performance liquid chromatography
ICP-AES	inductively coupled plasma - atomic emission spectroscopy
ICP-MS	inductively coupled plasma - mass spectrometry
IR	infrared spectroscopy
ITEX	in-tube extraction
IUPAC	pure and applied chemistry
K_d	diffusion constant
K_{fs}	a fiber coating/sample matrix distribution constant
L/S	liquid-to-solid ratio
LC-GC	liquid chromatography-gas chromatography
LC-MS/MS	liquid chromatography-mass spectrometry/mass spectrometry
LLE	liquid-liquid extraction
$LogP_{ow}$	partition octanol-water logarithm
LOV	lab-on-valve
LPME	liquid-phase microextraction
M.P.	melting point
$M.W_t$	molecular weight
MEPS	microextraction by packed sorbent
MIPs	molecular imprinted polymers
MISPE	molecularly imprinted solid-phase extraction
MRLs	maximum residue limits
MS	mass spectrometry
μSPE	micro solid phase extraction
MWCNTs	multi-walled carbon nanotubes
n	the number of moles extracted by the coating
NRC	national research council
OCI	on-column injector
OCPs	organochlorine pesticides
OSP	on-line sample preparation

LIST OF ABBREVIATIONS (CONT.)

OX	oxalate
PA	polyacrylate
PAHs	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
PDMS	polydimethylsiloxane
PLE	pressurized liquid extraction
POPs	persistent organic pollutants
PROSPEKT	programmable on-line solid phase extraction
PTFE	polytetrafluoroethylene
PTV	programmable temperature vaporizer
RAM	restricted access materials
SBSE	stir bar sorptive extraction
SDME	single drop microextraction
SDU	solvent delivery unit
SES	sequential extraction schemes
SFOE	solidified floating organic drop microextraction
SIA	sequential injection analysis
SI-MC	sequential injection microcolumn extraction
SI-SFC	sequential injection stirred-flow cell extraction
SI-SPE	sequential injection solid phase extraction
SLME	supported liquid membrane extraction
SM&T	standards, measurements and testing program
SPE	solid-phase extraction
SPME	solid-phase microextraction
SVE	solvent vapour exit
SWCNTs	single-walled carbon nanotubes
TCLP	toxicity characteristic leaching procedure
TWA	time-weighted average
USEPA	united stated environmental protection agency
V_f	the fiber coating volume
V_s	the sample volume

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CHAPTER 1

INTRODUCTION

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INTRODUCTION

1. Process analytical chemistry

The aim of process analytical chemistry is to provide both quantitative and qualitative information about a chemical process. Such information can be used not only to monitor and control a process, but also to optimize its efficient use of energy, time, and raw materials. Consequently, while saving costs and minimizing effluent release the product quality and consistency is improved.

Flow injection analysis (FIA) and sequential injection analysis (SIA) are techniques which are well suited for automation of traditional wet chemical methods. Because of their reliability, robustness and miniaturization of the entire analytical setup, flow techniques have found their path in the laboratory and as continuous process analytical methods. As a result of the increasing degree of automation in the chemical industry together with increasing demand of continuous effluent control by authorities, the concept of process analytical chemistry emerged within the traditional field of analytical chemistry [1]. Under sometimes very harsh conditions, with chemical and mechanical stresses, the analyzing equipment should deliver precise results at a 24/7 operation mode. The reproducibility of the analytical method is of greater importance than the absolute accuracy, because the calibration interval can be less frequent and most often changes in the process are of greater interest of process control reasons. Sampling is a key factor in process analytical chemistry, regardless if the sampling is done manually or automatically. No analytical method can compensate for non-representative samples [2].

In the concept of process analytical chemistry, five different ways of coupling the analytical process with the analyzer should be distinguished and will be later used in this dissertation for method development exploiting flow-based approaches. These are off-line, at-line, on-line, in-line and non-invasive methods (Fig. 1). The first two methods, off-line and at-line methods are distinguished by the requirement of manual removal of the sample and transport to the measuring instrument. In off-line methods, the sample is analyzed in a centralized facility with sophisticated, and perhaps even automated instrumentation (Fig. 1a). The advantages of this

approach include the economy and efficiency of time-sharing and availability of an expert staff for consultation, methods development, and maintenance. The disadvantages, which include the delay between submission of sample and reporting of results, the competition among users for the resources, have led to a second method that is at-line method. In this type of analysis a dedicated instrument is installed in close proximity to the process line (Fig. 1b). The advantages include faster sample processing, closer control of the analysis by the personnel, and employment of a simpler instrument with less cost and maintenance, and greater ease of use.

In on-line methods, an automated sampling system is used to extract the sample, condition it, and present it to an analytical instrument. Hereby the response time is very short allowing the operators to adjust process parameters before the quality goes out of given specifications. On-line methods can be divided into two subcategories (Fig. 1d): continuous and intermittent methods. In continuous on-line methods, a sample stream is continuously directed through the analytical instrument. Most gas analyses based on IR absorption, chemiluminescence, fluorescence and colorimetric methods belongs to this category. Intermittent methods require discrete sampling and a continuous method that permit the sample to flow continuously through the instrument. The analysis is performed by an automatic instrument, but the main difference compared to the continuous methods is that the result is not obtained continuously, but at discrete times. Gas chromatography, titrations and flow injection analysis are examples of intermittent on-line methods.

In-line methods are based on chemical analysis done in-situ, inside the process line, using a probe that is chemically sensitive (Fig. 1c). The in-line methods enable continuous monitoring of the process without any time delays. Such methods are for example pH and other ion selective electrodes. Disadvantages of in-line methods including difficulties of keeping the probe clean and making calibrations and maintenance.

Finally, the non-invasive methods include those methods where the probe or instrument does not need to be in physical contact with the sample. Hereby, the sometimes very difficult problems associated with process sampling can be omitted. This method obviously has a great deal in common with remote sensing and non destructive analysis.

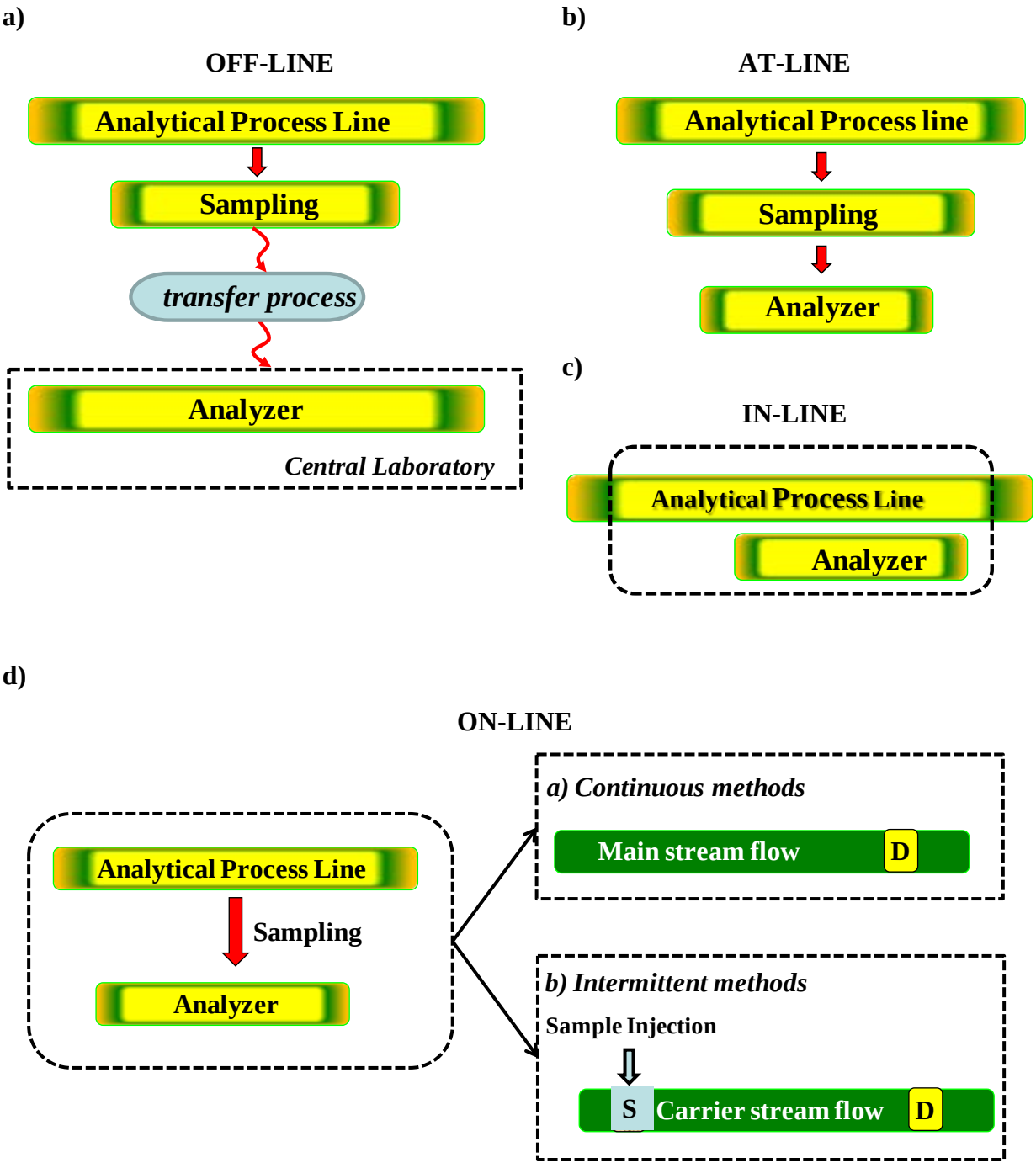


Figure 1. Coupling of the analytical process with analyzer: a) off-line, b) at-line, c) in-line, d) on-line methods, (S: sample, D: detector).

2. Sample preparation techniques

In cases where the analytes of interest are present in complex matrices of, for example, environmental or biological origin, the sample is usually not suitable for direct introduction into the analytical instruments [3]. Sample preparation is aimed to transform target species to a form and concentration suitable for analysis and it is often considered to be a critical step in the analytical procedure [4] because it not only helps to achieve the low detection limits set by regulatory authorities by cleaning up the sample matrix but also acts to preconcentrate analytes of interest from a dilute sample matrix to a level of detection by an instrument of choice. In most of the analytical procedures, sample preparation is very often the slowest and the most costly part of the analytical process particularly when multi-step procedures are utilized which takes about 50-75% of the total time of the analysis [3]. For this reason, the faster this procedure can be done, the more quickly the analysis will be completed. This procedure must be highly reproducible and without appreciable loss of the analytes. The ideal of sample preparation method should involve a minimum number of working steps, be easy to learn, be environmentally friendly according to green chemical principles and be economical [5]. Further, as the number of samples grows high-throughput and fully automated analytical techniques becomes required.

2.1 Sample preparation techniques for the determination of organic pollutants in environmental samples

Several sample preparation techniques are available that offer different degrees of selectivity, enrichment capability, speed of operation and convenience to the analyst and environment. Modern techniques of sample preparation address the need for the reduction of organic solvent consumption, miniaturization, automation and ultimately on-site, in-situ or in-vivo sampling. Currently used sample preparation methods for assays of organic pollutants include liquid-liquid extraction (LLE), liquid-phase microextraction (LPME), solid-phase extraction (SPE) and solid-phase microextraction (SPME). These approaches are usually easy to carry out and call for optimization of several parameters to enhance the performance of the overall analysis. Criteria for choosing a suitable method includes the physico-chemical properties of analytes, available time and equipment, specificity, and sensitivity. In general, several approaches are possible depending on the analytical problem.

2.1.1 Liquid-liquid extraction (LLE) and liquid-phase microextraction (LPME)

Liquid-liquid extraction (LLE) is based on the partition of organic compounds between the aqueous sample phase and an immiscible organic solvent, which is non- or just slightly polar. Hexane and cyclohexane are frequently used for compounds with aliphatic moieties, whereas dichloromethane and chloroform are popular solvents for non- to medium-polar contaminants. After the extraction step concentration down to a few milliliters by rotary evaporation is performed.

Advantages are the simplicity of the procedure and inexpensive equipment (mostly glassware). Numerous methods have been developed for almost any analyte. Disadvantages include contamination and loss of sample (by adsorption to the glassware) due to several sample handling steps. Large volumes of solvents (sometimes chlorinated) are used and have to be disposed of. Solvents with high purity have to be used in trace analysis contributing to the high costs of the analyses. Usage in the field is not easy and methods are usually performed off-line in a manual or semi-automated mode [6]. Nowadays, LLE methods [7] are more and more replaced by sorptive extraction techniques (such as solid phase extraction (SPE) with advanced materials and solid phase microextraction (SPME), see next section) because they require minimal handling and consumption of organic solvents as well as offer high selectivity and enrichment factors.

Analysts performing LLE have experienced difficulties such as exposure to large volumes of organic solvents, formation of emulsions, and generation of mountains of dirty. To address these problems, scaling-down the size of LLE is more applicable than scaling-up, which is critical for productive preparation. In this context, miniaturization of LLE techniques has led basically to new methodologies i.e., single drop microextraction (SDME) [8-10], supported liquid membrane extraction (SLME), hollow-fiber liquid phase microextraction (HF-LPME) [9-12], dispersive liquid-liquid microextraction (DLLME) [9,10,13-15] and solidified floating organic drop microextraction (SFOE) [8-10] with extremely reduction of extraction solvent volume. The developments and advantages of these methods have been discussed in some comprehensive reviews published lately [3,16,17].

Representative examples of miniaturized systems for LPME including flow approaches, chip-based technology and 96-well robotic stations are given below. A cost-effective automatic flow-based system with on-line optical detection based on multicommutation and exploiting

the liquid-liquid extraction methodology has been published recently to meet green chemical principles [18]. The schematic diagram of the system is shown in Fig. 2.

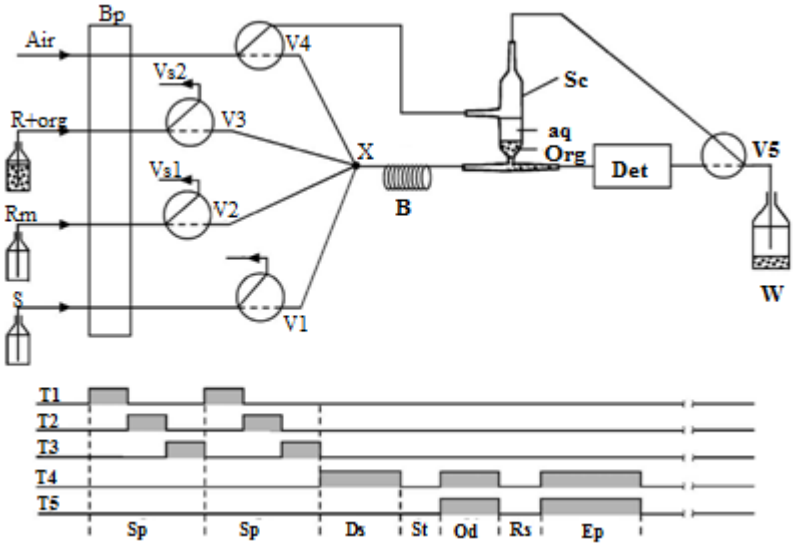


Figure 2. Diagram of the flow-based analysis system. S: sample, Rm: reagent; V1, V2, . . ., V5: three-way solenoid valves; B: reaction coil, Sc: glass separation chamber, Det: spectrophotometer, Vs1, Vs2 and Vs3: storing vessels; BP: peristaltic pump; x: joint device; W: waste. T1, T2, . . ., T5: valves timing course, Sp: sampling cycle, Ds: sample zone displacing step, St: phases separation step, Od: organic phase displacing step, Rs: signal reading step, and Ep: separation chamber emptying step. The shadow surface beneath of lines indicated that the associated valve was switched on [18].

A LPME system on a glass chip was proposed for continuous solvent extraction, phase separation, and detection of atropine in synthetic urine, and of atropine and scopolamine in standard pharmaceuticals [19] but also for separation of Fe [20]. To this end, an aqueous Fe complex (Fe-4,7-diphenyl-1,10-phenanthroline-disulfonic acid) and a chloroform solution of capriquat (tri-*n*-octylmethylammonium chloride) were introduced separately into a microchannel (250 mm) to form a parallel two-phase laminar flow producing a liquid-liquid aqueous-organic interface (Fig. 3). The authors noted that in the microchannel, the aqueous-organic interface did not attain the upper-lower arrangement produced by differences in specific gravity normally observed in LLE. The extraction system required no mechanical stirring, mixing, or shaking.

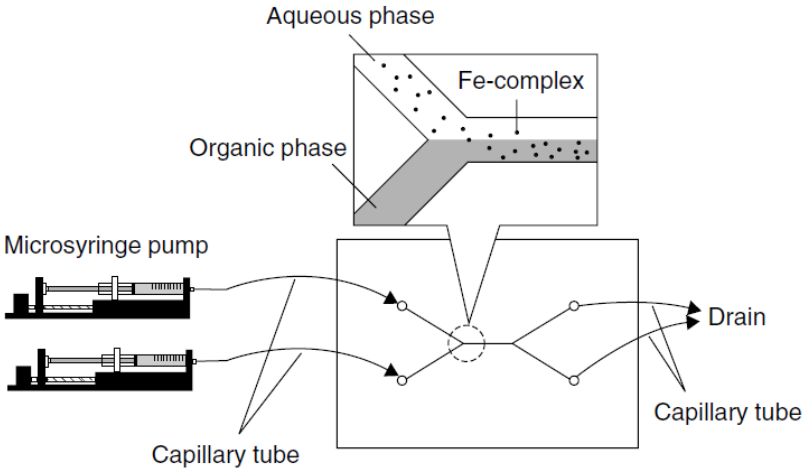


Figure 3. Schematic diagram of microextraction system on a glass chip [20].

Fully automated 96-well plate LPME extraction was reported by Peng *et al*. [21-23] in the last decade for processing of biological samples. They exploited the efficiency of 96-channel, programmable, robotic liquid handling workstation technology to automate methodology for this LLE variation. A LPME plate was prepared by adding inert diatomaceous earth particles to a 96-well plate with hydrophobic GF/C glass fiber bottom filters. Samples and solvents were added to the plate sequentially (Fig. 4). LPME occurred in the interface between the two liquid phases and on the surface of individual particles in each well plate. The organic phase extracts were eluted under gentle vacuum into a 96-well collection plate. The approach was used for initial purification of combinatorial library samples and for quantitative analysis of carboxylic acid-based matrix metalloprotease inhibitor compounds in rat plasma [23].

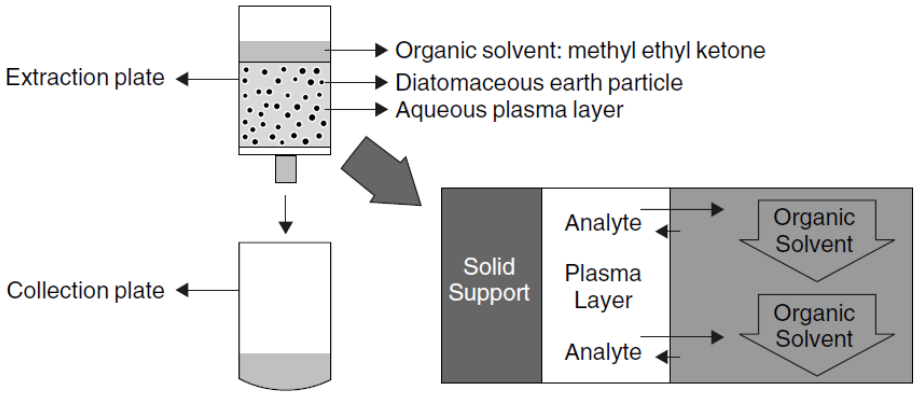


Figure 4. Schematic representation of automated LPME on a well plate [23].

2.1.2 Solid phase extraction (SPE)

Solid phase extraction (SPE) is a sample preparation technique based on principles similar to those of HPLC and is employed for the selective sorption of analytes of interest from liquid matrices. This transfer of analytes from the liquid sample matrix to the solid sorbent is influenced by the selection of appropriate conditions of the liquid matrix and the sorbent according to the physico-chemical properties of the analytes.

A typical SPE protocol involves four steps; conditioning, loading, washing, and elution (Fig. 5) and defined in the following:

i. *Conditioning* - the sorbent is wetted with a suitable solvent to activate the functional groups on its surface. This step may be followed by an equilibration step whereby the wetting solvent is replaced by water.

ii. *Loading* - the sample is percolated through the sorbent.

iii. *Washing* - interfering components of the matrix are removed while taking care not to elute the analytes as well.

iv. *Elution* - analytes of interest are eluted with an appropriate solvent and further preconcentration takes place by evaporation with N_2.

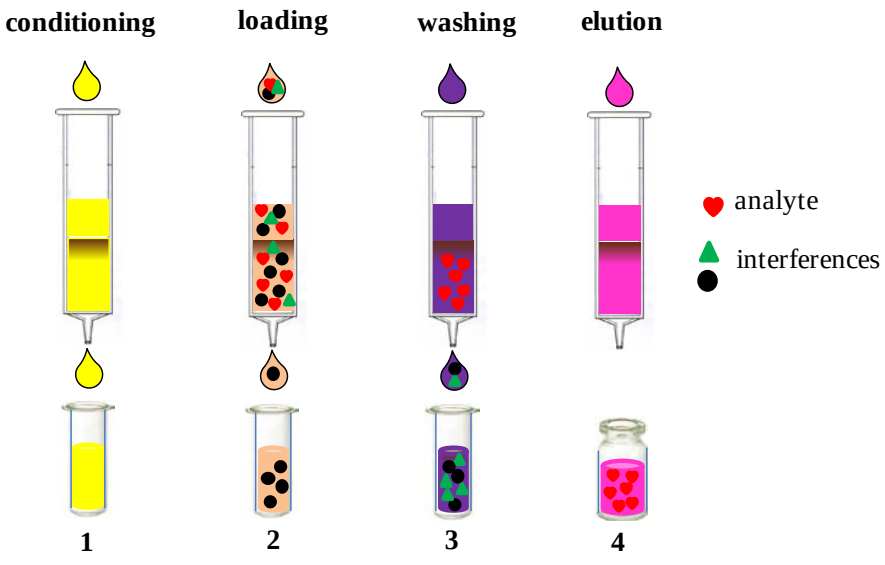


Figure 5. Conventional steps of manual SPE procedures

Several factors influence the efficiency of the SPE process but the two most important are retention and capacity. Retention of analytes on the sorbent should be maximum during the loading and washing steps but minimal during the elution step. To understand these, knowledge of the hydrophobic, polar and/or ionogenic properties of both the analytes and sorbent are required. The most common retention mechanisms in SPE are based on van der Waals forces (non-polar interactions), hydrogen bonding, dipole-dipole forces (polar interactions) and cation-anion interactions (ionic interactions). As a result, separation on SPE sorbents can be referred to as reversed phase, normal phase or ion exchange, respectively.

Reversed phase separation

Reversed phase separation involves a polar or moderately polar sample matrix (such as water) and a non-polar sorbent. The analyte of interest is usually mid- to non-polar (such as pesticides), retention of the organic analytes onto the sorbent is mainly due to the attractive weak forces between the carbon-hydrogen bonds in the analyte and the functional groups on the sorbent surface (van der Waals forces). A non- to mid- polar (less polar than the sample matrix) solvent is then employed to disrupt the interaction between the analyte and sorbent.

Sorbent materials based on carbon, chemically modified silica and polymers have been employed as reversed phase SPE sorbents. Carbon-based media consist of graphitic, non-porous carbon with a high affinity for organic polar and non-polar compounds. Two types of graphitized carbon sorbents, graphitized carbon blacks and porous graphitic carbons, are commercially available for SPE applications. Retention of analyte is based primarily on the structure of the analyte rather than on the interactions of functional groups of the analyte with the sorbent surface. Bonded-silica is currently the most commonly used solid phase for reversed phase SPE. Reversed-phase bonded silica sorbents having alkyl groups covalently bonded to the silica gel backbone interact primarily with analytes via van der Waals forces (Fig. 6). Bonded silica sorbents are commercially available with many variations in the organic ligand group (R). Common bonded phases produced for reversed-phase applications include hydrophobic, aliphatic alkyl groups, such as octadecyl (C_{18}), octyl (C_8), ethyl (C_2), or cyclohexyl, covalently bonded to the silica gel backbone. Aromatic phenyl groups can also be attached. The R ligand can contain cyanopropyl or diol hydrophilic functional groups that result in polar sorbents used in normal-phase applications. Ionic functional groups, including carboxylic acid, sulfonic acid, aminopropyl, or quaternary amines, can also be bonded to the silica sorbent to produce ion exchange sorbents. Polymeric sorbents (e.g. Amberlite XAD

resin) are based on cross-linked polystyrene divinylbenzene materials. These sorbents are used for the retention of hydrophobic compounds that have some hydrophilic functionality, especially aromatic compounds. Analytes are normally eluted with mid- and non-polar solvents since the polymeric material is stable in almost all matrices. Silica materials can be coated or bonded with hydrophilic polymers. The pores of the polymer allow small hydrophobic organic compounds such as drugs to reach the silica surface while large molecules such as proteins are prevented from reaching the silica surface and can then be washed off the sorbent (so-called restricted access materials, RAM). The capacity of SPE columns can be determined by recording breakthrough curves.

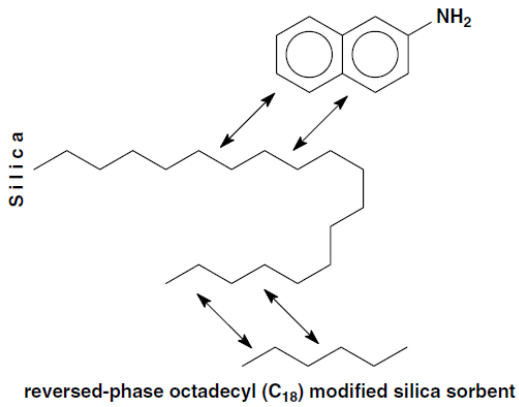
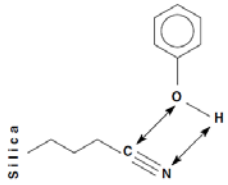


Figure 6. Interactions between analytes and non polar bonded silica sorbents *via* van der Waals forces.

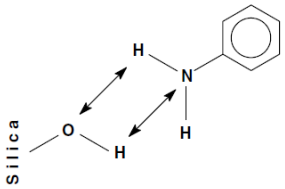
Normal phase separation

Normal phase separation involves a polar analyte, a mid- to non-polar matrix (e.g. acetone, chlorinated solvents and hexane) and a polar sorbent. Retention of an analyte under normal phase conditions is primarily through interactions between polar functional groups of the analyte and polar groups on the sorbent surface via hydrogen bonding, dipole-dipole interactions, π-π interactions and induced dipole-dipole interactions. The elution solvent should be one that is more polar than the sample matrix.

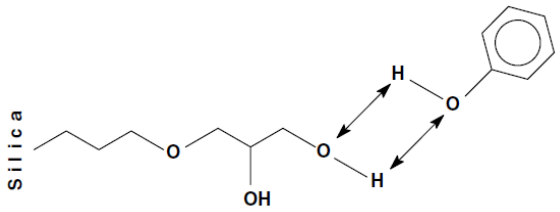
The most common polar sorbents used for normal-phase SPE are silica $(SiO_2)x$, alumina (Al_2O_3), magnesium silicate ($MgSiO_3$ or Florisil), and the bonded silica sorbents in which silica is reacted with highly polar functional groups to produce aminopropyl $[(SiO_2)_x$-$(CH_2)_3NH_2]$-, cyanopropyl $[(SiO_2)_x$-$(CH_2)_3CN]$-, and diol $[(SiO_2)_x$-$(CH_2)_3OCH_2CH(OH)$-$CH_2(OH)]$-modified silica sorbents (Fig.7). Bonded normal phase silica sorbents have short alkyl chain with polar functional groups (free hydroxyl groups) bonded to the surface.



(*a*) cyanopropyl-modified silica sorbent



(*b*) silica sorbent



(*c*) diol-modified silica sorbent

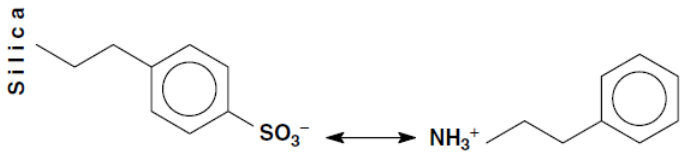
Figure 7. Interactions between analytes and polar sorbents *via* dipolar attraction or hydrogen bonding.

This causes them to be more hydrophilic compared to bonded reversed phase silica sorbents, hence they are used to adsorb polar compounds from non-polar matrices. Polar SPE sorbents are often used to remove matrix interferences from organic extracts of plant and animal tissue.

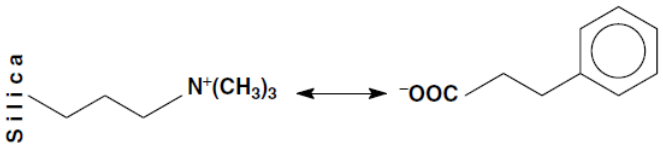
Ion-exchange separation

Ion exchange SPE is used for compounds that are charged when in solution. SPE sorbents for ion exchange are available based on either polar polymeric resins or bonded silica sorbents. Ion-exchange sorbents contain ionized functional groups such as quaternary amines or sulfonic acids, or ionizable functional groups such as primary/secondary amines or carboxylic acids. The charged functional group on the sorbent associates with the oppositely charged counterion through an electrostatic, or ionic bond (Fig. 8).

The functional group on the sorbent can be positively or negatively charged. When the sorbent contains a positively charged functional group and the exchangeable counterion on the analyte in the liquid sample matrix is negatively charged, the accumulation process is called *anion exchange*. Conversely, if the functional group on the sorbent surface is negatively charged and the exchangeable counterion on the analyte in the liquid sample matrix is positively charged, the accumulation process is called *cation exchange*.



(*a*) benzenesulfonic acid-modified silica sorbent



(*b*) trimethylaminopropyl-modified silica sorbent

Figure 8. Interactions between analytes and ion-exchange sorbents (a) strong cation exchange sorbent and (b) strong anion exchange sorbent.

Anion-exchange sorbents for SPE contain weakly basic functional groups such as primary or secondary amines which are charged under low-pH conditions or strongly basic quaternary ammonium groups which are charged at all pHs. Cation-exchange sorbents for SPE contain weakly acidic functional groups such as carboxylic acids, which are charged under high-pH conditions, or strongly acidic aromatic or aliphatic sulfonic acid groups, which are charged at all pH levels. ‘‘Weakly’’ acidic or basic ion-exchange sorbents are pH dependent because they dissociate incompletely, while ‘‘strongly’’ acidic or basic ion-exchange sorbents are pH independent because they dissociate completely. Thus, the control of pH is essential in ion-exchange SPE to ensure that the analyte of interest is charged during the loading and washing steps to enable its retention on the sorbent. In addition, the analyst has to bear in mind that silica based sorbents are only stable within the pH range of 2 to 8. The bonded phase can be hydrolysed and cleaved off the silica surface or the silica itself can dissolve at pH levels below 2 or above 8 for prolonged times.

SPE sorbents are commercially available in four formats: contained within cartridges, in columns fashioned like syringe barrels, in well plates, or in disks (Fig. 9) [24-26].

The scope of SPE is very wide with notable applications in the clean-up of biological fluids for the extraction of drugs [27]. The disposable cartridges reduce the handling of body fluids such as urine, and blood and hence the biohazard to the operator is minimized. The second widespread application of SPE has been used in environmental samples such as river water

[28,29] and sewage effluents [30] where large quantities of very dilute samples have to be extracted. In the extraction of pesticides from water, SPE efficiency depends on factors such as the nature of the water sample due to presence of particulate matter, presence of interfering compounds such as surfactants as well as the ionic strength and sorbent treatment.

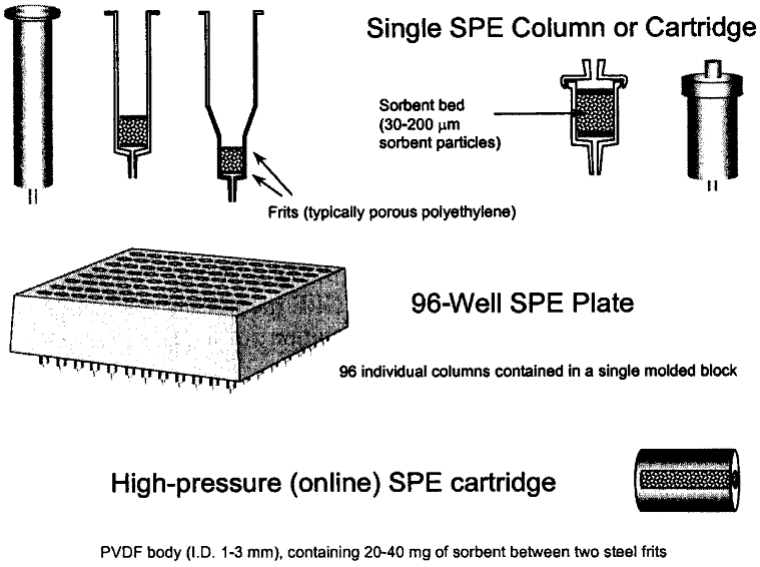


Figure 9. Schematic illustration of SPE formats [24]

SPE is especially suitable for the analysis of aqueous samples and can be performed on- or off-line methods. With liquid samples extraction and preconcentration can be performed at the same time and with vacuum manifolds batches with up to 24 samples can be prepared simultaneously. The SPE cartridge introduced important features such as standardization, greater reproducibility and a wider range of phases to choose from. Although the cartridges are usually single-use and disposable, the overall cost of SPE is lower than the cost of solvents and the manpower needed for traditional LLE.

SPE has several advantages over liquid-liquid extraction (LLE) in that it: (i) requires less organic solvent than LLE, (ii) provides higher selectivity due to the wide range of sorbent chemistries available, (iii) does not involve the formation of emulsions as in LLE, (iv) yields cleaner extracts, (v) provides higher and more reproducible recoveries, (vi) achieves higher sample throughput, and (vii) is easily automated.

2.1.2.1 Molecularly imprinted solid-phase extraction (MISPE)

Molecular imprinted polymers (MIPs) are synthetic materials with artificially generated recognition sites able to specifically rebind a target molecule in preference to other closely related compounds as an attempt to synthesize antibody mimics. These materials are obtained by polymerising functional and cross-linking monomers around a template molecule, leading to a highly cross-linked three-dimensional network polymer. The monomers are chosen considering their ability to interact with the functional groups of the template molecule. Once polymerisation has taken place, template molecule is extracted and binding sites with shape, size and functionalities complementary to the target analyte are established (Fig. 10).

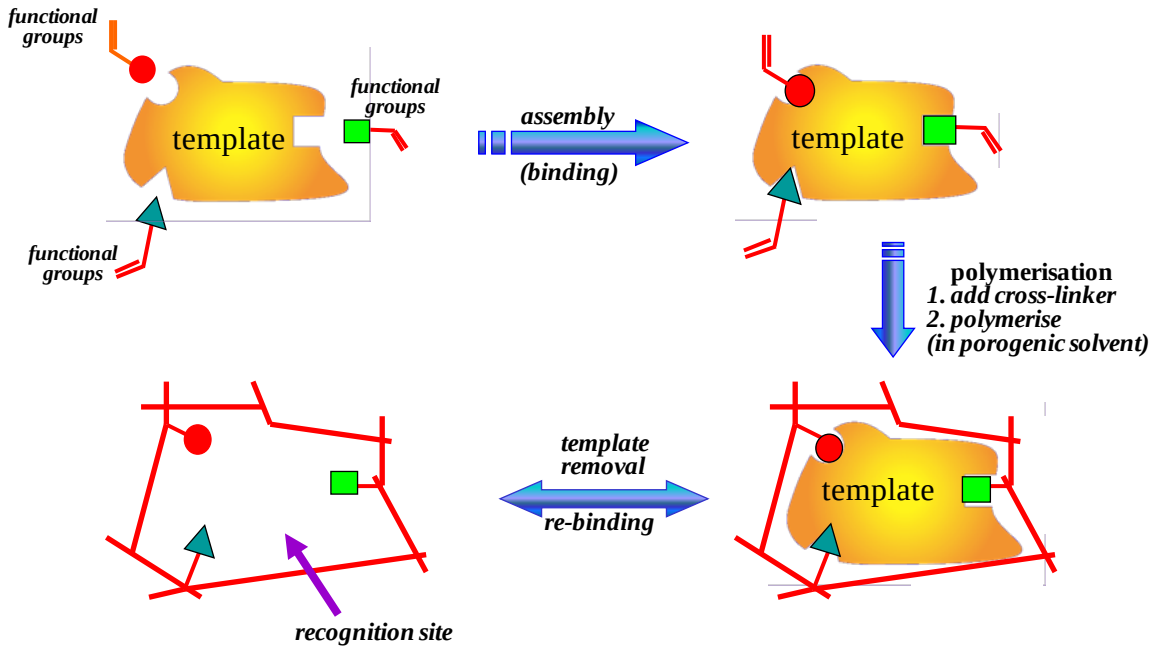


Figure 10. Schematic depiction of the preparation of molecular imprints.

Three different approaches to prepare MIPs have been reported [31]: covalent, semi-covalent, and non-covalent approaches. The covalent approach involves the formation of reversible covalent bonds between the template and monomers before polymerization. Then, the template is removed from the polymer by cleavage of the corresponding covalent bonds, which are reformed upon rebinding of the analyte. The high stability of template-monomer interaction leads to a rather homogenous population of binding sites, minimizing the existence of non-specific sites. However, this approach is rather restrictive since it is not easy to design an appropriate template-monomer complex in which covalent bond formation and cleavage are readily reversible under mild conditions.

An intermediate option is the semi-covalent approach. In this case, the template is also covalently bound to a functional monomer, but the template rebinding is based only on non-covalent interactions.

The non-covalent approach is based on the formation of relatively weak non-covalent interactions (i.e. hydrogen bonding, polar interactions) between the template molecule and selected monomers before polymerization. This approach is widely used due to its experimental simplicity and to the commercial availability of different monomers able to interact with almost any kind of template. However, it is not free of some drawbacks derived from the fact that the template-monomer interactions are governed by an equilibrium process. Thus, in order to displace the equilibrium to form the template-monomer complex, a high amount of monomer is used. Consequently, the excess of free monomers is randomly incorporated to the polymeric matrix leading to the formation of non-selective binding sites.

Nowadays MIPs use in SPE, so-called *molecularly imprinted solid-phase extraction (MISPE)*, is by far the most advanced technical application of MIPs. The MIPs offer the advantages of an easy, low cost, rapid preparation, and high thermal and chemical stability although early analytes breakthrough might be an issue [32]. In addition, increased selectivity relative to other sorbents produces because larger sample volumes can be extracted. MIPs have recently been proven to have high chemical robustness, providing the opportunity to clean and reactivate them for multiple uses in SPE specially for the analyte in the presence of complex biological or environmental matrix interferences.

Retention of the analyte on these sorbents is due to shape recognition, but other physico-chemical properties including hydrogen bonding, ionic interactions, and hydrophobic interactions are important to retention as well. However, desorption is usually more difficult if any sorbent has increased affinity for the analyte. One problem noted in MISPE is incomplete removal of the template molecule from the polymer, resulting in leaching of the analyte (bleeding) during subsequent analyses. Stringent cleaning of the sorbent and analytical confirmation of the lack of interfering compound can reduce this problem. The use of MIPs as selective sorbent materials allows performing a customized sample treatment step prior to the final determination. This is of special interest when the sample is complex and the presence of interferences could prevent final quantification by typical chromatographic techniques coupled to common detectors. Due to the inherent selectivity provided by MIPs,

yesteryears have seen a growing interest in this area and it has been extensively reviewed [31,33-35].

2.1.2.2 Carbon nanotubes solid-phase extraction (CNT-SPE)

Carbon nanotubes (CNTs) represent the novel carbon-based nanomaterials with unique properties such as high surface areas, large aspect ratios, remarkably high mechanical strength as well as high electrical and thermal conductivities. They can be described as a graphite sheet rolled up into a nanoscale-tube. Two structural forms of CNTs exist single-walled (SWCNTs) and multi-walled (MWCNTs) carbon nanotubes. CNTs length can be as short as a few hundred nanometers or as long as several micrometers. SWCNTs have diameters between 1 and 10 nm and are normally capped at the ends. In contrast, MWCNTs diameters are much larger (ranging from 5 nm to a few hundred nanometers) because their structure consists of many concentric cylinders held together by van der Waals forces (Fig. 11).

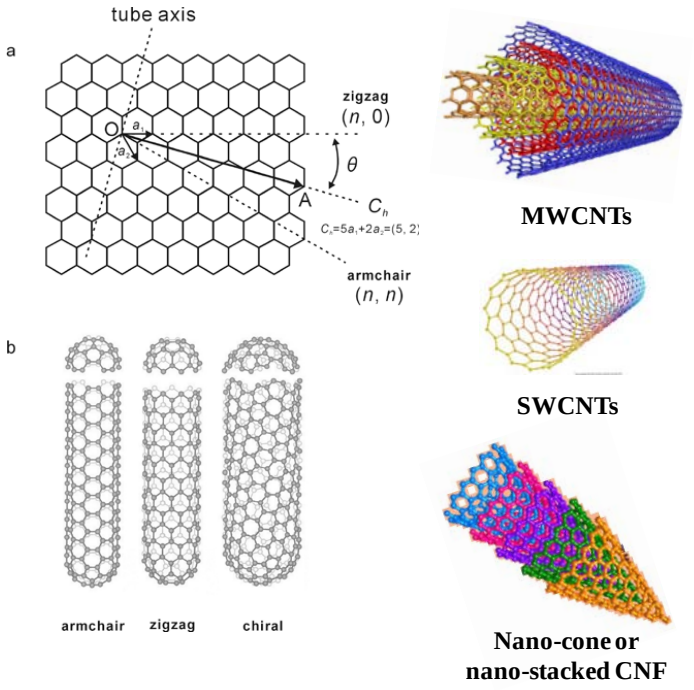


Figure 11. Carbon nanotubes structures; single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs) and nano-stacked or nano-cone carbon nanofibers, CNF: carbon nanofibers.

It is known that oxidation of carbon surfaces can offer not only more hydrophilic surface structure, but also a larger number of oxygen-containing functional groups, which increase the

ion-exchange capability of the carbon material. Gas phase oxidation of carbon nanostructures increases mainly the concentration of hydroxyl and carbonyl surface groups, while oxidation in the liquid phase increases particularly the content of carboxylic acids. The amount of carboxyl and lactone groups on the CNTs treated with nitric acid was proven to be higher in comparison to the process conducted using H_2O_2 and $KMnO_4$ [36].

The characteristic structures and electronic properties of carbon nanotubes allow them to interact strongly with organic molecules, *via* non-covalent forces, such as hydrogen bonding, π-π stacking, electrostatic forces, van der Waals forces and hydrophobic interactions. These interactions along with their hollow and layered nanosized morphology make them a good candidate for application as a SPE sorbent [37].

Recent applications of carbon nanotubes for removal and enrichment of herbicides in different types of samples are summarized in reviews [38-40]. Carbon nanotubes with high porosity and large adsorption area seems to be good for solid phase microextraction (SPME) coating as well. Rastkari et al. [41] proposed a novel SPME coating by attaching CNTs onto a stainless steel wire through organic binder. The results showed that the CNTs fiber exhibited higher sensitivity and longer life span (over 150 times) than the commercial carboxen/poly-dimethylsiloxane coating.

Zhou et al [42] compared the trapping efficiency of CNTs and C18 packed cartridge using sulfonylurea herbicides as the model compounds. When the matrices of the samples were very simple, such as tap water and reservoir water, the enrichment performance between these two adsorbents had no significant difference. However, carbon nanotubes become much more suitable to extract herbicides from complex matrices (such as seawater and well-water). Carbon nanotubes could be also used in a format of disk [43]. Incorporating sorbents of small particle size, the disk format possesses a larger surface area than the cartridge, resulting in good mass transfer and fast flow rates [44]. To enhance the sorption capacity of the disks, double or even triple disks have been used together. A comparison study showed that the double-disk system (comprising two stacked disks with 60 mg of CNTs) exhibited extraction capabilities that were comparable to those of a commercial C18 disk with 500 mg sorbent for nonpolar or moderately polar compounds. The triple layered CNTs disk system showed good extraction efficiency with sample volumes up to 3000 mL [45].

2.1.2.3 Mixed-mode and multi-dimensional SPE

Each of the types of SPE sorbents discussed above retains analytes through a primary mechanism, such as by van der Waals interactions, polar dipole-dipole forces, hydrogen bonding, or electrostatic forces. However, sorbents often exhibit retention by a secondary mechanism as well. Bonded silica ion-exchange sorbents primarily exhibit electrostatic interactions, but the analyte also experiences nonpolar interaction with the bonded ligand. Nonpolar bonded silicas primarily retain analytes by hydrophobic interactions but exhibit a dual-retention mechanism, due to the silica backbone and the presence of unreacted surface silanol (-SOH) groups [46]. Recognition that a dual retention mechanism is not always detrimental to an analysis has led to the design of mixed-mode sorbents. The development of mixed-mode sorbents and multi-dimensional approaches capitalized on multi-dimensional retention mechanisms has evolved as a logical extension of the observation of secondary interactions [47].

A mixed-mode sorbent is designed chemically to have multiple retentive sites on an individual bead particle (Fig. 12). These sites exploit different retention mechanisms by chemically incorporating different ligands on the same sorbent [26]. For example, sorbents have been manufactured that contain hydrophobic alkyl chains and cation-exchange sites on the same sorbent particle. Mixed-mode SPE sorbents are particularly useful for the extraction of polar organic analytes from biofluids [48].

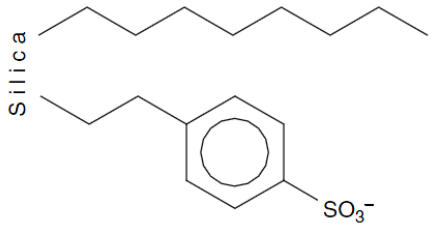


Figure 12. Example of a mixed-mode sorbent consisting of silica modified with octyl (C8) alkyl chains and strong cation-exchange sites bonded on the same sorbent particle.

Alternatively, there are several different mechanical approaches to achieving multi-dimensional mode retention (Fig. 13). Sorbent particles of different types (i.e., a hydrophobic sorbent and an ion-exchange sorbent) that exhibit separate mechanisms of retention can be homogeneously admixed, or blended, in the same column, or they can be layered into the same column by packing one phase over another [49]. Additionally, multi-dimensional phases can

be stacked by arranging in tandem series sorbents of different retention mechanisms contained in separate columns. The technique of stacking or sequencing sorbents in tandem columns, termed *chromatographic mode sequencing* (CMS), can afford very selective isolation of analytes [50,51].

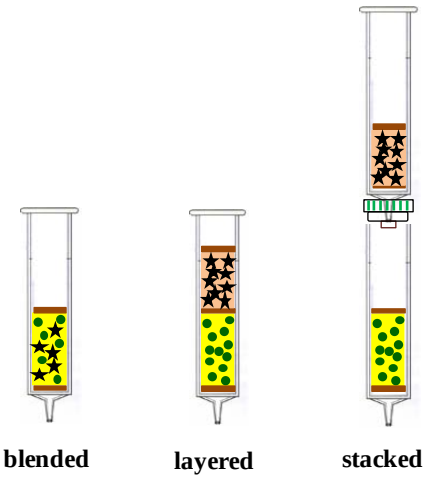


Figure 13. Multi-dimensional SPE mode approaches.

2.1.2.4 Automatic and on-line SPE methods

2.1.2.4.1 Sequential injection SPE (SI-SPE)

Sequential injection analysis (SIA) was introduced by Ruzicka and Marshall [52] to overcome the limitations of flow injection analysis (FIA) in terms of fluidic handling, reagent consumption and fixed architecture. SIA is based on continuous bi-directional pumping of carrier and reagent streams as precisely coordinated and controlled by a computer [53-55]. A typical SIA manifold is illustrated in Fig. 14.

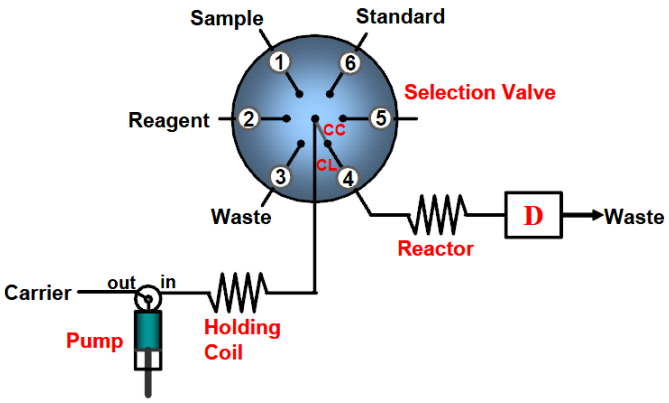


Figure 14. A typical SIA manifold. CC: central communication conduit, CL: central communication line, D: detector.

A basic SIA manifold involves a multi-position selection valve, furnished with a central communication conduit (CC) that can rotate to address each one of the peripheral ports of the valve, a central communication line (CL) which, *via* a holding coil (HC), is connected to a syringe pump. The ports of the selection valve are coupled to reservoirs of sample, reagents, detector and other peripheral units (e.g., a sorbent column), respectively. A typical operational procedure is described as follows; firstly the CC is directed to the port connected to the sample line and a well defined volume of sample zone is aspirated into the HC. Then, the valve is redirected to the port connected to the reagent line and a reagent zone is aspirated into the HC adjacent to the sample zone. Afterwards, the selection valve is turned to the port connected to the detector, and the sample and reagent zones are propelled forward through the reaction coil where zone dispersion occurs, resulting in the formation of detectable species which subsequently is monitored by the detector.

The most notable advantage of SIA is the drastic reduction in the consumption of sample and reagents, hence resulting in less waste production which is more and more important nowadays due to the increasing costs in the disposal of chemical wastes. In addition, the accurate handling of sample and reagent zones is readily controlled by a computer within the single-channel manifold, allowing full automation. It is easy to reprogram the method and shift from one application to another. By employing solvent resistant materials for the conduits, SIA system can virtually handle any kind of reagents.

However, there are two limitations in the operation of “primitive” SIA systems. The first one is that since sample and reagents are stacked one after another in the HC and only two adjacent zones in the HC can disperse into each other and thus facilitate the reaction, it is generally difficult to accommodate more than two reagents with the sample. In practice, this limitation has been eliminated by the hybrid flow injection/sequential injection techniques in which additional reagents are added downstream by auxiliary syringe pumps [56,57]. The second one is the limited operating capacity associated with the use of syringe pumps, although this seldom presents itself as a problem. Although SIA is an established techniques for performing solution chemistry, its most significant potential lies in that it offers versatile schemes for the more complicated on-line sample manipulation steps (e.g. SPE methods) before the actual measurement [55,58]. Thus, the ports of the multiposition selection valve can be coupled to various units including reservoirs, detectors, pumps, reactors, separators, special cells, and other manifold, as illustrated in Fig. 15.

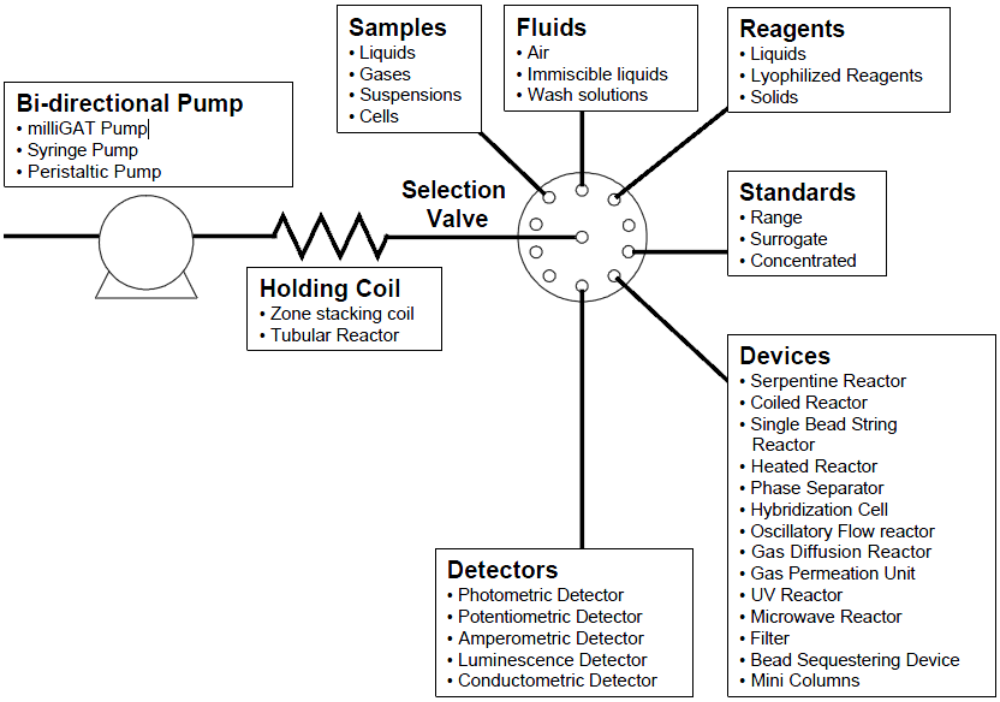


Figure 15. Potential of SIA for automated sample pretreatment [59].

Column-based solid phase extraction (SPE), which employs an appropriate solid material, is among the most efficient and widely employed separation/preconcentration techniques in SIA [53]. This sample processing method has been growing rapidly as a consequence of its straightforward operation and high separation and preconcentration capabilities. Depending on the nature of the measurement and on the retention mechanism, various extraction materials have been employed. Moreover, methods of extraction in PTFE knotted reactors [56], where the tubing of the reactor serves as the extraction material, can also be considered as belonging to the family of SPE methods. A comprehensive review on automatic SIA methods incorporating solid-phase reaction has been published lately [60]. Readers interested in the role of FIA for SPE assays are directed to the following monograph [61]. SPE material in conventional FIA or SIA systems is generally permanently packed in a column which is treated as an integral component of the manifold prior to detection of the analyte and for reliable application, the break-through capacity of the column, the column dimension and the particle size of the sorbent material must be carefully balanced. Although smaller particles sizes result in higher break-through capacities, finer particles tend to cause progressively tighter packing and hence create flow resistance in the column. As opposed to flow injection analysis systems, on-line SI-SPE analyzers are less prone to the build-up of flow resistance

due to the discontinuous flow of solutions through the packed reactor, accurate control and individual programming of the flow rates for each stage of the analytical protocol, and the likelihood of applying bi-directional flow approaches whenever back-pressure effects are detected. However, the repeated use of sorbent reactors in the flow network might give rise to several problems. Some sorbent materials undergo volume changes, i.e., swelling or shrinking, at different conditions. Malfunction of the reactive surface of the sorbent occurs as a consequence of irreversible changes such as contamination, deactivation of the surface or even loss of active sites. Moreover, incomplete elution of the retained species from the sorbent medium leads to carry-over effects between consecutive runs. A superb alternative to overcome those mentioned drawbacks is the surface renewal scheme, the so-called *bead injection (BI)* analysis [62].

2.1.2.4.2 Bead injection (BI)

Bead injection (BI) is a novel approach to assays based on liquid-solid interaction which combines the advantages of solid-phase chemistry in a miniaturized mode (μSPE) with the novelty of fluidic handling of microcarrier beads in flow systems, allowing automated surface renewal and post-analysis manipulations. Surface renewal is an especially critical feature because bead surfaces become contaminated or otherwise dysfunctional with repeated used. Bead injection solid phase extraction (BI-SPE) technique is based on the microfluidic manipulation of a precise volume of suspended beads that serve as a solid-phase carrier for reagents or reactive groups. The injected bead suspension is trapped at a strategic location in a flow manifold, where it is subsequently perfused by the analyte solution, buffers, or auxiliary reagents. Chemical reactions occur at the bead surfaces and can be analyzed in real time, either directly on the solid phase (solid-phase optosensing) or within the eluting liquid phase [63-65]. A multiparameter approach is also possible, by monitoring simultaneously the changes in the solid and liquid phases. At the end of a measurement cycle, the beads can be automatically discarded, collected, or rerouted if desired.

BI can be seen as the third generation of flow injection micro-analytical techniques, following the development of SIA. Indeed, SIA is the perfect vehicle for BI, which in turn enhances SIA by eliminating the problem of mixing reactants during the loading process. Other advantages of BI include improved assay sensitivity, because the use of surface-bounded reagents minimizes sample dilution; lower limits of detection because the analyte can accumulate on the beads; expanded analysis schemes using parallel and/or orthogonal detection of analyte(s)

in the bead and/or solution phases and compatibility with a wide range of instruments including spectrophotometric and electrochemical detectors.

The BI procedure comprises five steps, as shown in Fig. 16a.

Step 1; an exact volume of a bead suspension is aspirated through the selection valve *via* a syringe pump and loaded into a flow cell as beads are trapped into a distinct geometry.

Step 2; the beads are perfused with a buffered carrier stream, and the baseline for subsequent measurement (spectroscopic or electrochemical) is established.

Step 3; a sample is injected, and the analyte is trapped on the bead surfaces.

Step 4; this analyte is treated with auxiliary reagents or eluted from the bead column.

Step 5; the spent beads are automatically discarded from the flow cell at the end of the assay cycle.

On-bead detection typically requires reestablishing the signal baseline, rendering the beads "invisible" to the detector. Maintaining a constant baseline during the measurement cycle is crucial. In general, reproducible BI analysis requires consistency in the bead packing and the mechanical and/or chemical stability of the bed layer. This can be achieved with a well-designed flow cell and prudent selection of fluidic control parameters on an SIA system as shown in Fig. 16b

The flow cell must trap the beads in a geometry that allows uniform perfusion and, preferably, simultaneous monitoring of the entire bead layer. This can be achieved by retaining beads within a specialized flow cell called *a jet ring cell*, which consists of a tube positioned perpendicular to a flat surface, leaving a narrow circular gap (see Fig. 16a). The liquid can escape through the gap in a radial fashion, while the beads are retained and perfused. At the end of the analysis cycle, the jet ring cell is emptied, and the spent beads are removed from the detection zone.

New trends in this field are directed to couple BI with the new generation of miniaturized flow systems, the so-called *Lab-on-valve (LOV)*. First we will introduce the fundamental principle of LOV followed by its potential in executing reliable BI assays.

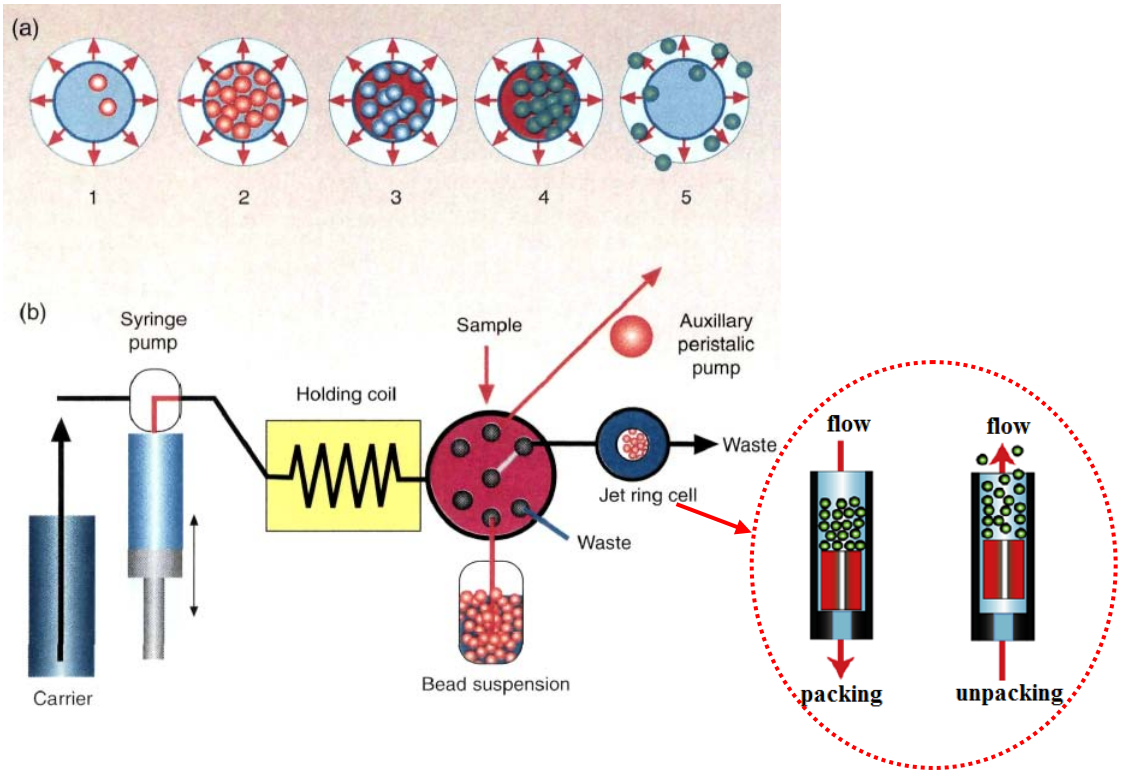


Figure 16. Jet ring cell and SI system. (a) A narrow gap in the ring traps the beads and creates a jet. The resulting carrier flow travels radially from the jet, as shown in the experimental protocol; (1) the beads arrive and are trapped, and (2) a baseline is established during the wash period. The beads are (3) exposed to a sample solution, and (4) either the sample is eluted, or auxiliary reagents are perfused. At the end of the analysis, (5) the beads are discarded, (b) An SIA system modified for BI. Typically, the system uses a 1-mL syringe, a 2-uL jet ring cell, and 20-500 uL of injected sample and/or reagent [66].

2.1.2.4.3 Lab-on-valve (LOV)

Lab-on-valve (LOV) was introduced by Ruzicka in 2000 [67] as a supplement for SIA manifold. Besides the common components of SIA systems (a multi-position selection valve, a holding coil and a syringe pump), an integrated microconduit, which is normally fabricated by hard PVC or more chemically resistant Ultem, furnished with microchannels corresponding to the ports of selection valve, is mounted atop of the multi-position selection valve. A basic LOV unit and LOV manifold for μSPE are depicted in Fig. 17 and 18, respectively. As the name implies, LOV is actually extended to constitute a small laboratory, potentially allowing a multitude of unit operations for a given assay to be executed in an on-line fashion. The LOV can be operated within a wide range of sample and reagent expenditure, from as low as microliter and submicroliter levels to normal ranges that are employed in conventional FIA/SIA operations. As a result of its versatility LOV may contain solid column reactors packed with small beads furnished with active groups and even detection facilities. In-valve detection by UV/Visible spectrometer or fluorometer is feasible in the LOV manifold, using

optical fibers which are affixed at the two ends of the flow cell/microcolumn. One of the fibers is used to direct the light from a light source into the LOV while the other one serves to guide the transmitted light or fluorescence to the detection device [68], as illustrated in Fig. 19.

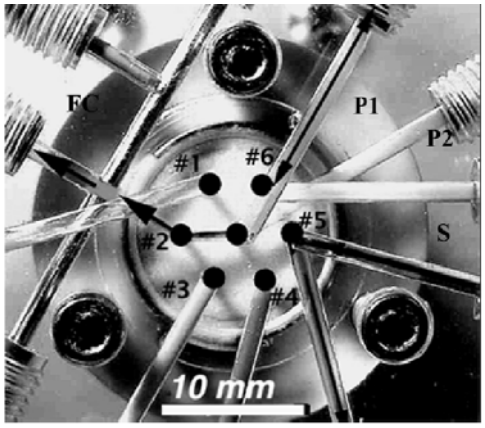


Figure 17. Lab-on-valve shown mounted atop a six position valve. P1, P2 are channels leading to holding coils and syringe pumps. Sample (S) is shown in a flow through sampling port (#5) that is connected to the sample container and peristaltic pump. The flow cell (FC) is shown in absorbance configuration using two optical fibers facing each other. Arrows leading from P1 through #2 and into the flow cell indicate the valve position during the transport and measurement of reaction product.

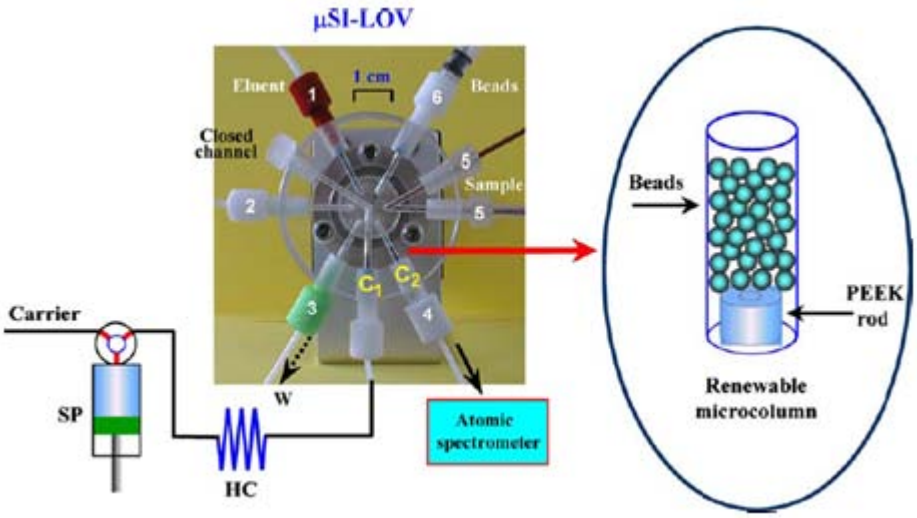


Figure 18. Diagram of a LOV system for bead injection (BI) incorporating two microcolumn positions (C1 and C2), along with a close-up of a packed renewable microcolumn [60].

The multiposition flow cell can be readily configured exactly as jet-ring-cell (see above), as illustrated in Fig. 20, where the change in bead optical properties can be monitored by absorbance (Fig. 20D), fluorescence (Fig. 20E) or reflectance (Fig. 20F) spectrometry.

These techniques are termed solid-phase optosensing [69] and involve detection of extracted compounds onto the beads. However, for other large detectors, such as AAS or ICP-MS, it is, of course, necessary to employ external detection devices. In this case, the LOV can serve as a front-end to modern instrumentation for introducing the pretreated measure and intelligently into the detector [70-72]. In addition, the operation of the selection valve and the syringe pumps are programmable and fully computer controlled.

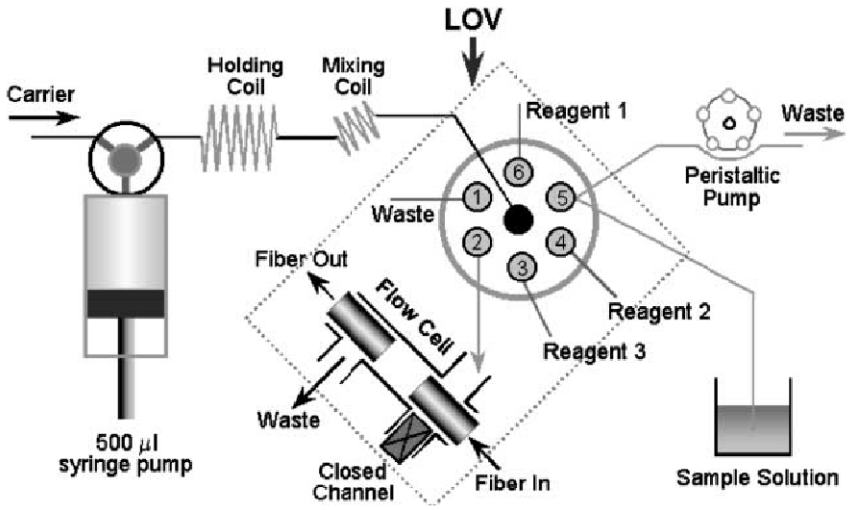


Figure 19. Schematic diagram of LOV microsystem incorporating a multipurpose flow cell configured for real time measurement of absorbance [73].

2.1.2.4.4 Bead injection- Lab-on-valve (BI-LOV)

BI-LOV approaches with renewable sorbents for preconcentration/separation have been developed for the determination of metal elements bioanalytical assays and affinity chromatography [64]. Whatever the bead material applied, the analysis cycle contains four stages: (i) first, a channel of LOV is packed with a small, well defined volume of bead material. Then, (ii) the packed channel is loaded with a well defined volume of sample, and the analyte or derivatized compound is retained on the column while the matrix goes to the waste. Thereafter (iii) the retained species is eluted by a small volume of an appropriate eluent, which subsequently is transferred to the detector. Alternatively, in-valve bead optosensing is possible by resorting to optical fibers integrated in the LOV platform. And finally (iv) the beads are discarded and new beads are aspirated for packing to perform the next cycle.

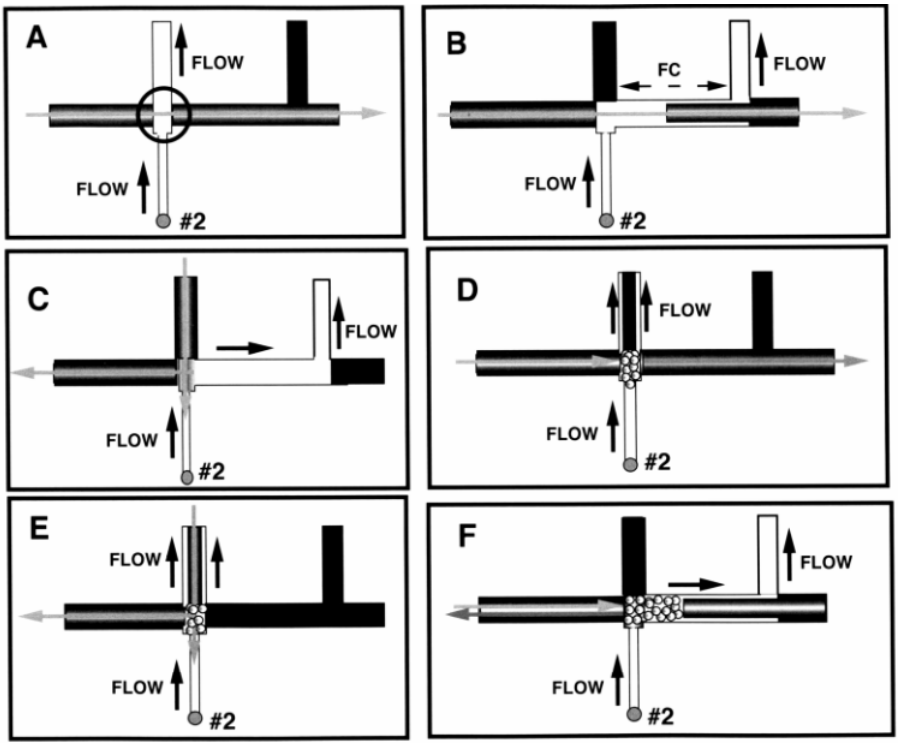


Figure 20. Multipurpose flow cell, which is integrated in LOV, uses optical fibers encased in stainless steel tubing that is proportioned to leave a 30 μm gap between the casing and channel walls. The fibers can be readily reconfigured for absorbance (A, B) and fluorescence (C) measurement. Since the 30 μm gap allows liquid to escape, but retains beads, the flow cell can also be assembled to a jet ring cell configuration for absorbance (D), fluorescence (E) and reflectance (F) measurement. Black blocks indicate filled and closed channels [67].

In order to handle the bead material reproducibly within the automatic system, it is imperative to ensure bead-size homogeneity and the spherical shape of the reagent-supporting entities so as to prevent compact settlement into the conduits of the flow set-up. Hence, chemically-modified silica-gel lumps are not really suited for this purpose as a result of their irregular shape and size distribution. However, bead material with a backbone of poly(styrene-divinylbenzene), poly(vinylpirrolidone) (e.g., Oasis-type beads) or agarose (e.g., Sephadex or Sepharose-type beads) fulfils the foregoing demands, inasmuch as they are perfectly spherical and have a uniform size. The use of either micellar media or ancillary continuous recirculation schemes for the bead suspension might be needed for reliable manipulation of hydrophobic sensing entities with higher density than water within the flow manifold [74].

Commercially available sepharose beads, mostly stored in 20% ethanol, can be used directly, while dry beads need to be suspended in an appropriate amount of water or buffer solution

before use. Hydrophobic sorbents are often wetted with organic solvent (ethanol) before dilution by water to obtain beads suspension within the range of 1:10-1:20 (m/v).

2.1.2.4.5 On-line coupling of SPE to column separation systems

1) On-line coupling of SPE with HPLC

A typical on-line SPE arrangement prior to HPLC separations is easy to perform in any laboratory using simple switching valves and commercial precolumns and their holders. This system is also named *column-switching liquid chromatography* [26] and involves intermittent sampling into HPLC (see Fig. 1d). The valve setup for on-line SPE is presented in Fig. 21. The SPE column is located in the loop position of the injection valve. The column-switching valve is used to direct the flow from the extraction column either to waste or to the HPLC analytical column. At the beginning of each run, the SPE column is conditioned. In the load position, sample is directly loaded onto the sorbent and then preconcentrated, while matrix components are removed during the washing step. The valve is then switched to the inject position, so that appropriate solution (normally HPLC mobile phase) can elute the analytes from the extraction column toward the analytical column, wherein they are separated prior detection. After elution, the valve is switched back to its original position to wash and re-equilibrate the extraction column.

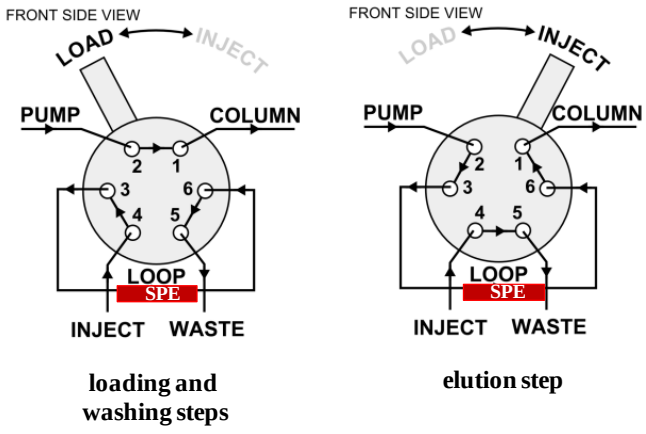


Figure 21. Schematic diagram of valves configuration for on-line SPE-HPLC system.

Hyphenated on-line SPE-HPLC systems are designed to improve not only sensitivity and selectivity of assays but also reduced sample manipulation and time, better intra- and inter-day reproducibility, and higher sample throughputs but typically require the use of program controlled switch valves and column reconfiguration for different analyses [75,76].

2) On-line coupling of SPE with GC

On-line coupling of SPE with GC is similar to that used for on-line LC-GC (see above). On-column, intercalated loop and programmable temperature vaporiser (PTV) interfaces are the main choice. An on-line technique [77,78] is show in Fig. 22, which combines sample preparation by means of solid-phase extraction (SPE) on a small precolumn packed with a hydrophobic phase, and capillary gas chromatography (GC) with mass spectrometric (MS) detection. The extraction of the concentrated analytes from the cartridge can either use a solvent or the elution can be accelerated by heating, effectively combining SPE and pressurized liquid extraction (PLE).

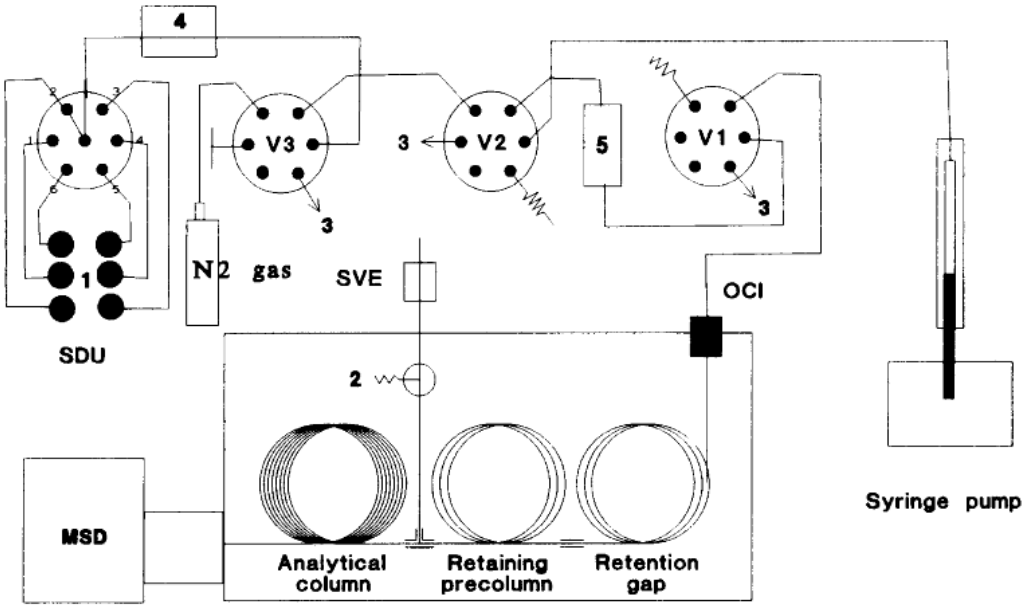


Figure 22. Scheme of an automated on-line SPE-GC system consisting of three switching valves (V1-V3), two pumps (SDU pump and syringe pump) and a GC system equipped with an SVE, and a mass-selective detector; 1, solvent channels; 2, purge leak restriction; 3, waste; 4, single-piston LC pump; 5, SPE precolumn; V1-V3, PROSPEKT valves; SDU, solvent delivery unit; SVE, solvent vapour exit; OCI, on-column injector [77].

2.1.2.4.6 Automatic SPE using disposable extraction cartridges

Several robotic SPE devices are commercially available (e.g., Prospekt , Prospekt-2 and OSP-2) which have the capability of using a fresh disposable SPE column for every sample. The brand name PROSPEKT is derived from the terms "PRogrammable On-line Solid Phase ExtraKTion" while the name OSP is abbreviated from "On-line Sample Preparation."

A unique feature of the Prospekt-2 robotic setup is its ability to perform thermally assisted solid-phase extraction controlling the temperature of the extraction cartridge may improve the performance of the overall extraction. Temperatures for loading, washing and eluting (desorption) may be manipulated. Typically, raw sample matrix (combined with internal standard) is utilized for injection onto the SPE cartridge, although sometimes an additional sample preparation step may precede this automatic analysis, *e.g.*, protein precipitation.

The Prospekt-2 is an integrated sample cleanup and injection system consisting of an Endurance TM (Spark Holland) autosampler for use also with microplates, LC pumps and the SPE cartridge system referred to as the ACE (Automated Cartridge Exchange) module, shown in Fig. 23. Samples are introduced by the autosampler. Solvents are delivered to the cartridge by a high pressure dispenser. Cartridge exchange and valve switching are performed by the ACE module. The analytes, now purified, are eluted from the SPE cartridge using column switching. The flow path is directed to the LC analytical column and then to the detection system *(e.g.,* mass spectrometer). The entire arrangement is controlled from a computer using the SparkLink™ software with Easy Access™ system control (Spark Holland). The Prospekt-2 uses two high pressure SPE cartridge clamps, two high pressure SPE solvent syringes and two SPE cartridge trays. In this configuration, elution is performed on one cartridge while the next cartridge is undergoing extraction. These short cycle times are ideal for keeping pace with LC-MS/MS analytical detection systems.

The SPE cartridge is able to withstand LC system pressures up to 300 bar and has a standard dimension of 10 x 2 mm. A full range of sorbent chemistries is available in the cartridge format, including the versatile Oasis HLB polymer sorbent (Waters) as well as several other polymer chemistries. Typical particle sizes used are 40 µm, although the HySphere™ cartridges (Spark Holland) specifically use a smaller particle size <10 µm. Sorbent mass per cartridge is typically either 20 or 45 mg, depending on particle chemistry. The standard capacity of the ACE module is 192 cartridges (two trays containing 96 cartridges each) an optional feeder mechanism allows access up to 960 cartridges (10 trays).

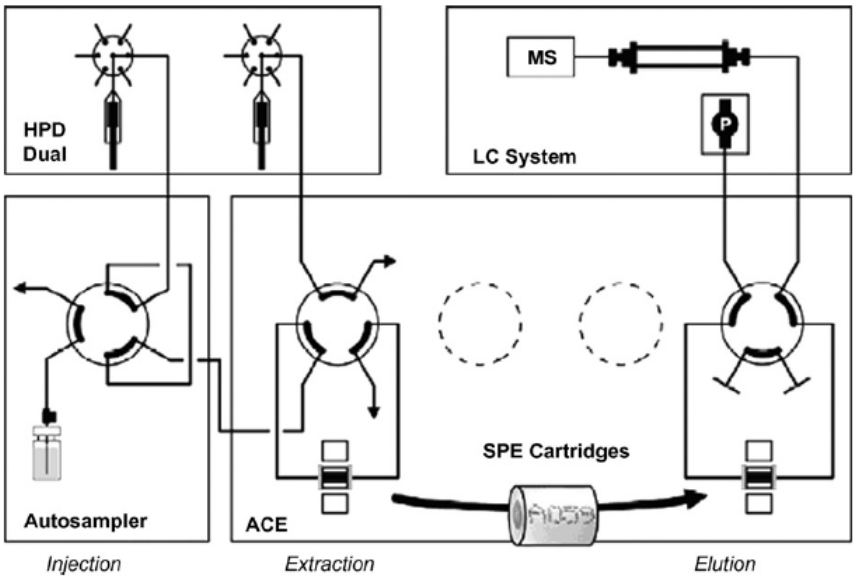


Figure 23. Schematic diagram of an on-line SPE-LC-MS system with Prospekt-2 device which is composed of an autosampler, a dual syringe high-pressure dispenser (HPD) and an automatic cartridge exchange (ACE) module [79].

2.1.2.4.7 Automatic SPE using traditional 96-well microplates

A special note is mentioned here of the capability for automatic solid-phase extraction offered by the SPE Twin PAL instrument (LEAP Technologies). Although the SPE Twin PAL does not technically match the Prospekt approach to fully enclosed on-line extraction and analysis, it does perform in a similar manner in an unattended, staggered parallel extraction mode. The SPE Twin PAL does not use individual SPE cartridges rather it processes standard 96-well solid-phase extraction microplates available from many vendors. The system uses positive pressure for liquid processing through the extraction wells. The eluate is collected in a clean microplate that is moved into place below the extraction plate processing area at the designated time. One syringe (typically 1-5 mL) is used for SPE plate conditioning, loading and washing, a second syringe (typically 100 µL) is used for injecting small volumes of eluate into the chromatographic system. Essentially, this LEAP approach bridges the gap between off-line SPE in microplates and on-line fully enclosed SPE.

2.1.2.4.8 Comparison of off-line versus on-line SPE methods

In general, off-line methods provide a larger flexibility, since they do not suffer from the constraint that the final elution conditions from the SPE cartridge need to be compatible with the detection system. In addition, other suitable means, for example gas chromatography (GC) or GC/MS, can be used for the final analysis. On the other hand, an important feature and

advantage of on-line SPE, compared with off-line SPE, is direct elution of the analyte from the extraction cartridge into the detection instrument. The time consuming off-line steps of evaporation, reconstitution, and preparation for injection into HPLC or GC are eliminated, making on-line SPE more efficient and fully automated (see Fig. 24). Since the entire volume of eluate might be analyzed, maximum sensitivity for detection is obtained.

Some other advantages of on-line SPE approaches are that: (i) samples and SPE cartridges are processed in a completely enclosed system protected against light and air, (ii) the operator is protected from working with hazardous and/or volatile organic solvents, and (iii) there is less handling and manipulation involved with no transfer loss of analyte.

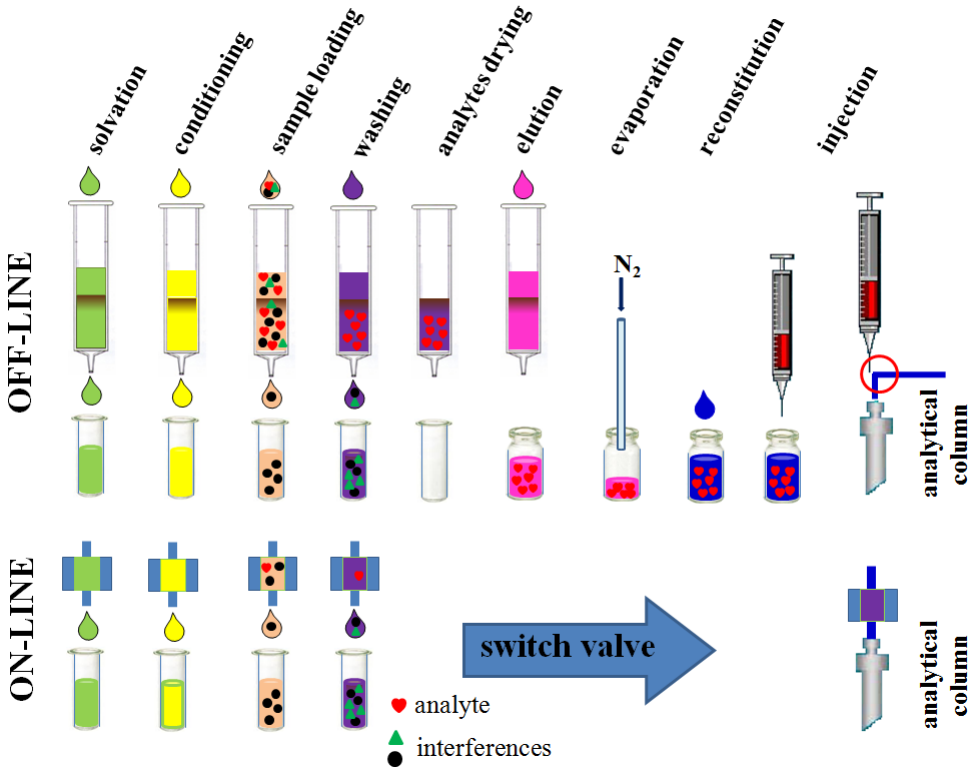


Figure 24. Schematic comparison of off-line versus on-line SPE coupled to HPLC.

2.1.3 Solid phase microextraction (SPME)

Solid phase microextraction (SPME) is a technique that was introduced in the early 1990s by Lord and Pawliszyn [80]. SPME is based on the establishment of equilibrium between the analyte and a fused silica fiber coated with a polymer (see Fig. 25) that can be liquid, solid or a combination of both. SPME is performed by exposing the fiber coated polymer to a sample matrix or its headspace until equilibrium is reached between the analyte partitioned on

the fiber coating and the analyte dissolved in the sample matrix. The concentration of the analyte extracted onto the fiber is proportional to its initial concentration in the sample matrix [80]. The analyte is then desorbed into a suitable separation and detection system, usually GC [81].

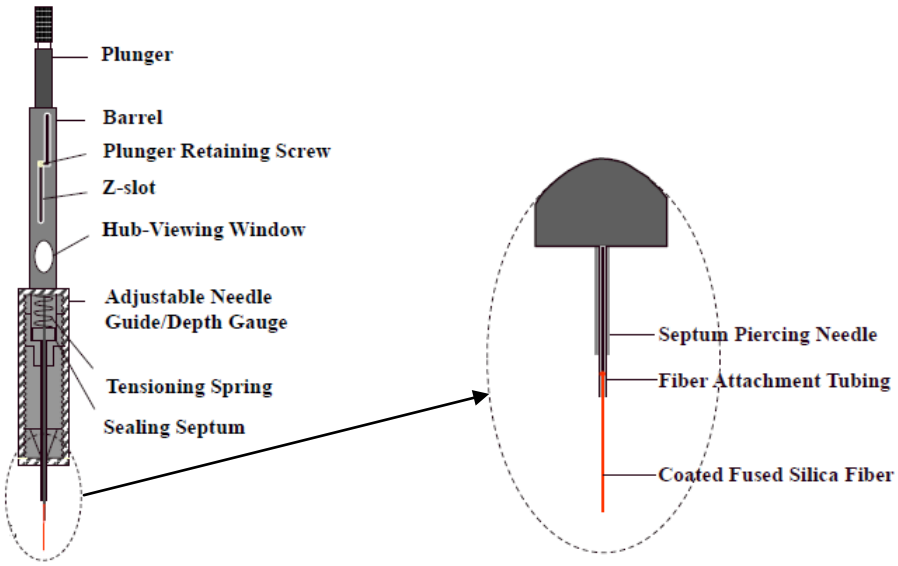


Figure 25. Commercial SPME device (Supelco)

The transport of analytes from the matrix into the coating begins as soon as the coated fiber has been placed in contact with the sample (Fig. 26). Typically, SPME is considered to be complete when the analyte concentration has reached distribution equilibrium between the sample matrix and the fiber coating. The equilibrium conditions can be described as [82]:

$$n = \frac{K_{fs} V_f V_s C_0}{K_{fs} V_f + V_s}$$

When n is the number of moles extracted by the coating, K_{fs} is the distribution coefficient between the fiber coating and the sample matrix, V_f is the fiber coating volume, V_s is the sample volume, and C_0 is the initial concentration of a given analyte in the sample.

Although various ways to implement SPME have been proposed, there are two primary approaches to conducting SPME (Fig. 27) with the sorbent coated on the outer surface of fibers or with the sorbent coated on the internal surface of a capillary tube. The fiber design can be interfaced with either GC or HPLC. However, the in-tube design has developed as an

easier approach for interfacing SPME with HPLC. The main reason for developing the alternative in-tube approach is to enhance sensitivity by using larger volume of the extraction phase (e.g. PDMS) and improving the kinetics of the mass transfer between sample and sorbent by increasing the surface to volume ratio of the extraction phase. The main disadvantage, however, is loss of the convenience associated with a syringe configuration, in particular for the introduction of the sample into the analytical instrument.

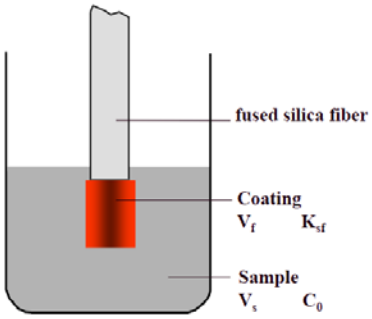


Figure 26. Microextraction with SPME, V_f: volume of fiber coating, K_{fs}: distribution coefficient between the fiber coating and the sample matrix, V_s: volume of sample, C_0: initial concentration of analyte in the sample.

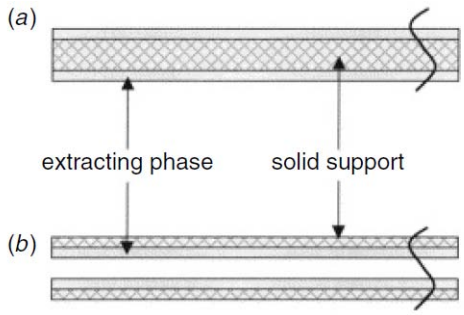


Figure 27. Two different implementations of the SPME technique: (a) polymer coated on outer surface of fiber; (b) polymer coated on internal surface of capillary tube.

The main advantages of SPME are the simplicity of operation, its solventless nature, and capability of analyte-matrix separation and analyte preconcentration [5,17]. SPME can be employed for field, in-situ, in-vivo and in-vitro sampling. The generally accepted limitations are the relatively poor reproducibility, lot-to-lot variations, lack of selectivity and tolerance to organic solvents and cost. In addition, the limited range of stationary phases that are commercially available restricts their use to hydrophobic compounds [83]. The commercially

available fiber coatings are polydimethylsiloxane (PDMS), divinylbenzene (DVB), polyacrylate (PA), carboxen (CAR-a carbon molecular sieve) and carbowax (CW-polyethylene glycol). The fibers are available in different coating combinations, blends or co-polymers, film thickness and fiber assemblies, thus widening the application fields.

Several variations of SPME are based on the geometry of the extraction phase such as coated fibers, vessels, stir bars, disks and coatings on the inside of tubes. The fiber design is the most convenient approach since analytes are easily desorbed from the fiber coating in the injection port of a GC [80]. Figure 28 illustrates the different configurations of SPME [84].

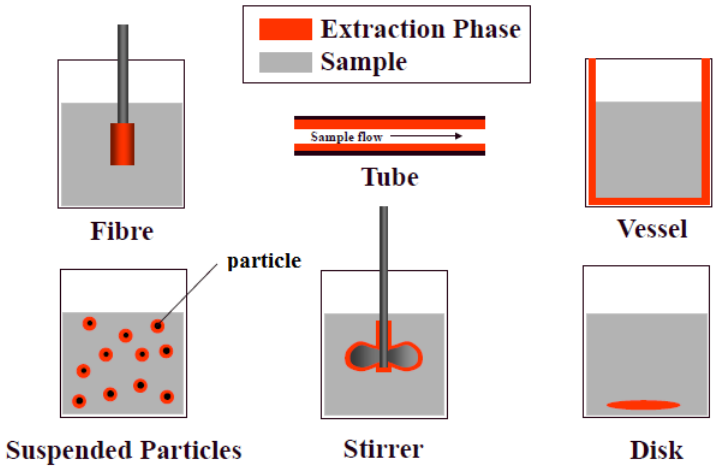


Figure 28. Configurations of SPME

2.1.3.1 Extraction modes with coated fiber

SPME can be performed in three modes; direct immersion, headspace and membrane protection modes. The different extraction modes are shown in Fig. 29.

2.1.3.1.1 Direct immersion SPME mode

The coated fiber is inserted directly into the sample matrix and analytes are transported to the fiber coating. Agitation may be employed to facilitate the transport of analytes from the bulk of the sample matrix to the vicinity of the fiber, resulting in rapid extraction. For aqueous matrices, agitation techniques such as fast sample flow, rapid fiber or vial movement, stirring or sonication may be employed. This is important to eliminate the formation of a “depletion zone” around the fiber as a result of fluid shielding and slow diffusion coefficients of analytes in liquid matrices. Agitation is not necessary for gaseous samples since the natural convection of air is sufficient for attainment of equilibrium in short periods of time.

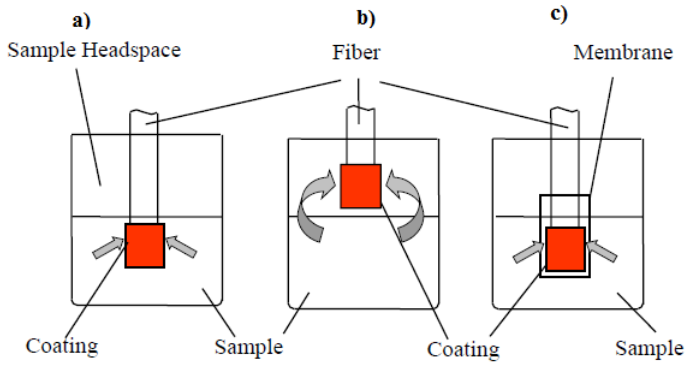


Figure 29. Fiber SPME modes with coated fiber: (a) direct extraction, (b) headspace SPME, (c) membrane-protected SPME

2.1.3.1.2 Headspace SPME mode

In this mode, analytes have to be present in the vapour phase before they can interact with the fiber coating which is suspended above the sample matrix. This mode protects the fiber from damage by high molecular and non-volatile species in the sample matrix such as humic acids and proteins. Headspace sampling also allows modification of the sample matrix parameters such as pH without affecting the fiber [85]. The choice of sampling mode has a significant impact on the extraction kinetics. For example, volatile analytes tend to be at a higher concentration in the headspace than in the liquid matrix and since diffusion rates in the gaseous phase are typically 4 orders of magnitude higher than in liquid media, equilibration times for volatiles are shorter in the headspace mode. Temperature and agitation also play an important role in headspace extraction kinetics in the sense that higher temperatures and agitation rates favor transport of analytes from the liquid matrix into the headspace [86].

2.1.3.1.3 Membrane protected SPME mode

The membrane functions to protect the fiber against damage when used for very dirty samples. A membrane made from an appropriate material may add some selectivity to the extraction process. The kinetics of membrane protected SPME are substantially slower than those of direct immersion since the analytes have to diffuse through the membrane before they can adsorb onto the coating. For example, Zhang and co-workers [87] covered a SPME fiber with a cellulose hollow membrane with a molecular weight cut-off of 18000 Da and were able to extract PAHs from complex aqueous samples containing humic acids. Their investigations showed that mass transfer rates increased when using elevated extraction temperatures.

2.1.3.2 Extraction modes with in-tube SPME

Another simple SPME construction is based on a piece of internally coated tubing. Figure 30 illustrates that there are two fundamental approaches for in-tube SPME; passive (static), when the analytes are transferred into the sorbent using diffusion (Fig. 30a) and active (dynamic), when the analytes are flowing through the tube (Fig. 30b). A coated tubing approach is useful in the design of passive sampling (static) devices discussed later, since in this case, the extraction rate is limited by the diffusion of analytes into the needle. In addition, active sampling (dynamic) is possible by heating and cooling of air contained in the upper part of the tubing, which causes movement of liquid or gaseous samples into and out of the tubing, facilitating mass transport of analytes from the sample to the coating.

2.1.3.2.1 Dynamic in-tube SPME

In this system we assume the use of a piece of fused-silica capillary, internally coated with a thin film of extracting phase (e.g. a piece of open tubular capillary GC column), or that the capillary is packed with extracting phase dispersed on an inert supporting material (a piece of micro-LC capillary column). In practice, in-tube SPME is implemented by replacing a section of the tubing in a commercially available autosampler, and then programming the autosampler to pass sample in and out of the extraction capillary until equilibrium or a suitable extraction level has been reached. Several options for implementing in-tube SPME which was also expanded to facilitate automation of sample preparation for HPLC are summarized in Fig. 31. This approach is limited to particulate-free gas and clean water samples. The headspace SPME approach can broaden the application of in-tube SPME. In that case, careful consideration to the mass transfer between sample and headspace should be given in order to describe the process properly.

2.1.3.2.2 Static in-tube SPME time-weighted sampling

In addition to the analyte concentration measurement at a well defined place in space and time, an integrated sampling is possible with a simple SPME system. This is particularly important in field measurements when changes of analyte concentration over time and place variations, must often be taken into account.

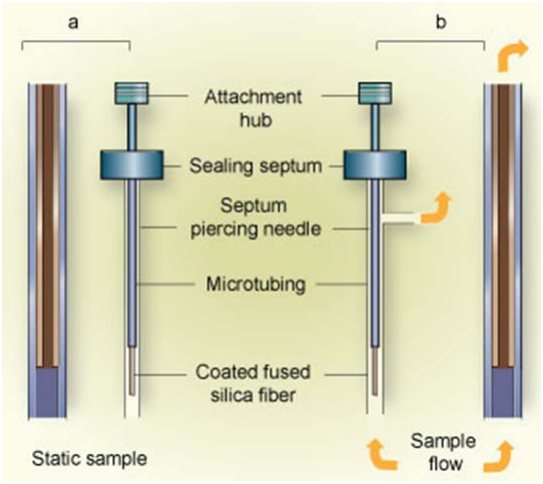


Figure 30. In-tube extraction SPME modes; (a) passive in-tube SPME (b) dynamic in-tube SPME.

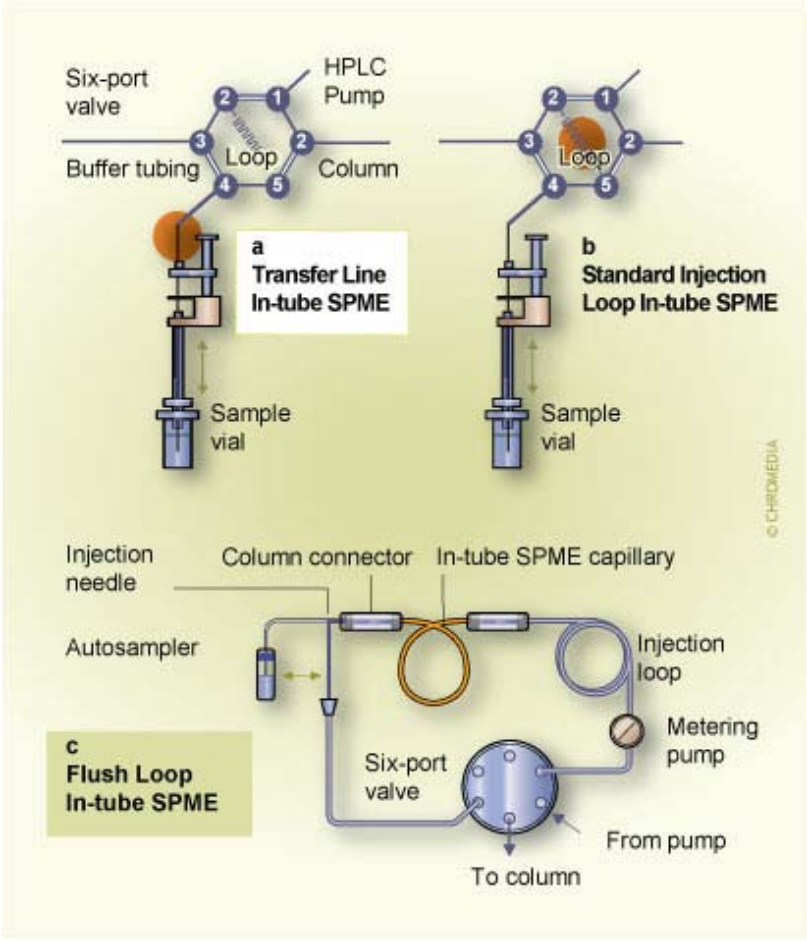


Figure 31. Different implementations of flow-through in-tube SPME. The position of the SPME capillary is highlighted with a solid dot in figures a and b.

When the extracting phase is not exposed directly to the sample, but is contained in a protective tubing (needle) without any flow of the sample through it (Fig. 30a), the extraction occurs through the static gas phase present in the needle. The integrating system can consist of extraction phase coating the interior of the tubing, or it can be an externally coated fiber withdrawn into the needle. These geometric arrangements represent a very powerful method able to generate a response proportional to the integral of the analyte concentration over time and space (when the needle is moved through the space). In these cases, the only mechanism of analyte transport to the extracting phase is diffusion through the gaseous phase contained in the tubing. During this process, a linear concentration profile (shown in Fig. 32a) is established in the tubing between the small needle opening, characterized by surface area (A) and the distance (Z) between the needle opening, and the position of the extracting phase.

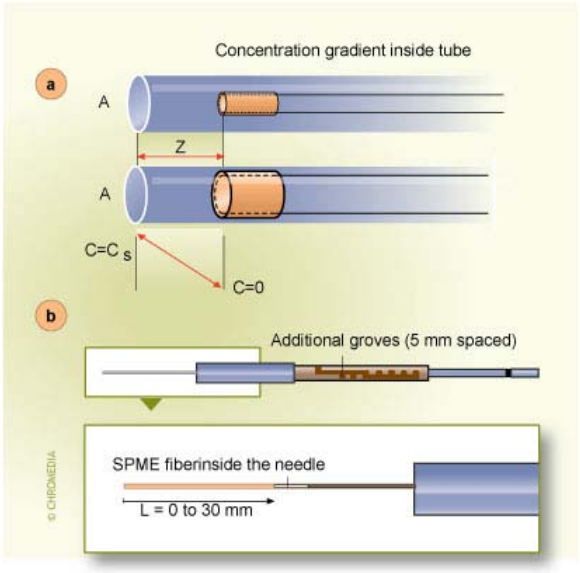


Figure 32. Use of SPME for in-tube time weighted average sampling. a) schematic, b) adaptation of commercial SPME fibers.

The exploitation of restricted access to the absorbing medium allows the implementation of SPME for time-weighted average (TWA) sampling. Where diffusion to the sorbent surface is limited, the sorbent can act as a sort of “zero sink” such that extraction is very far from equilibrium under normal sampling conditions. In practice then, any analytes reaching the sorbent surface are absorbed, essentially exhaustively [88]. The rate of distribution however, is still dependent on the sample concentrations over time, hence time-weighted average

sampling is achieved. This approach has been implemented to date with the conventional fiber assembly, by retracting the fiber a known distance inside the needle (Fig. 32b). The small size of the needle orifice limits diffusion to the sorbent surface, and the ultimate diffusion rate is also a function of the distance between the fiber tip and the end of the needle. Depending on the volatility and concentration of the analyte of interest, the fiber may be positioned either closer to or further from the end of the needle, to achieve the desired degree of non-equilibrium extraction and sensitivity. It would also be possible to implement this type of sampling with the sorbent coated on the interior wall of a capillary. To date however, the retractable needle implementation has gained the most attention, due to its ease of use and adjustability for the analyte and sample at hand.

2.1.3.3 Interface to analytical instruments

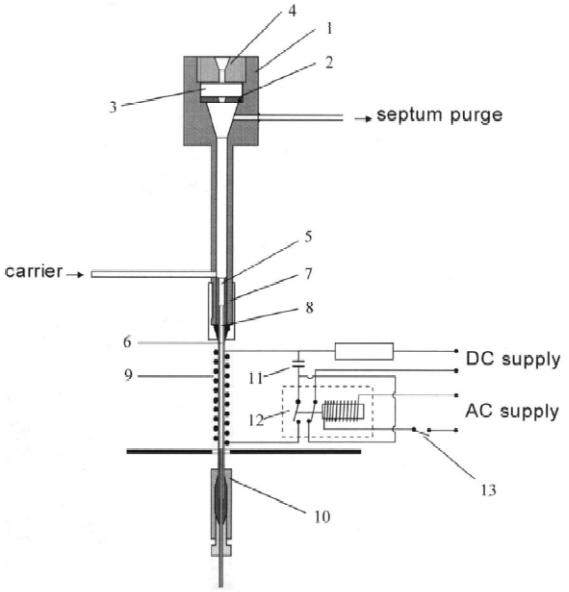
Because of its solvent-free nature, SPME can be interfaced conveniently to analytical instruments of various types. Most fiber SPME methods have been used in combination with GC [89] and LC [90-92] as well. A fiber SPME is allied to with GC and HPLC through a GC injector and a solvent desorption chamber (a part of injection loop), respectively, as detailed below.

2.1.3.3.1 SPME-GC

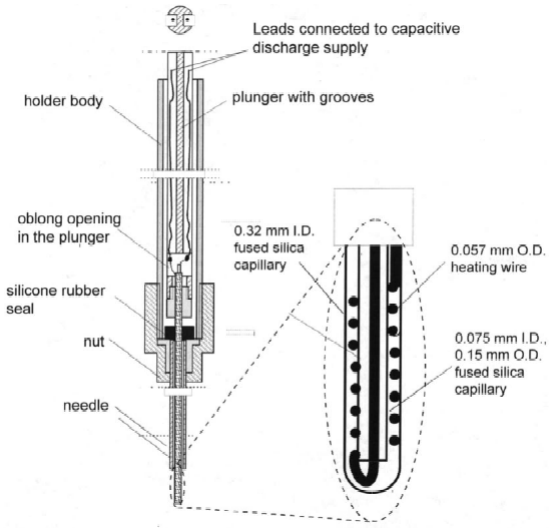
The standard GC injectors can be applied to SPME as long as narrow inserts with an inside diameter close to the outside diameter of the needle are used. The narrow inserts are required to increase the linear flow around the fiber, resulting in efficient removal of desorbed analytes. The split should be turned off during SPME injection. One way to facilitate sharper injection zones and faster separation times is to use rapid injection autosampling devices. An alternative solution is to use a dedicated injector, which should be cold during needle introduction, but which heats up very rapidly after exposure of the fiber to the carrier gas steam. A schematic diagram of such an injector is presented in Fig. 33a. Fiber can be also be designed to contain the heating element, as shown in Fig. 33b. In this case, no injector is necessary. The modified fiber can be introduced directly into the front of the column, and analytes can be desorbed rapidly by heating with a capacitive discharged current after the fiber has been exposed from the needle. Flash desorption injectors can be alternatively designed by passing a current directly through the fiber. This is possible if the rod is made of conductive material, as is the case with electrochemical SPME devices (Fig. 33c). When the electrical connection is made at the bottom of the interface, the fiber is rapidly heated by the discharging current.

The other option is to use laser energy to desorb analytes from the surface of fused-silica optical fiber.

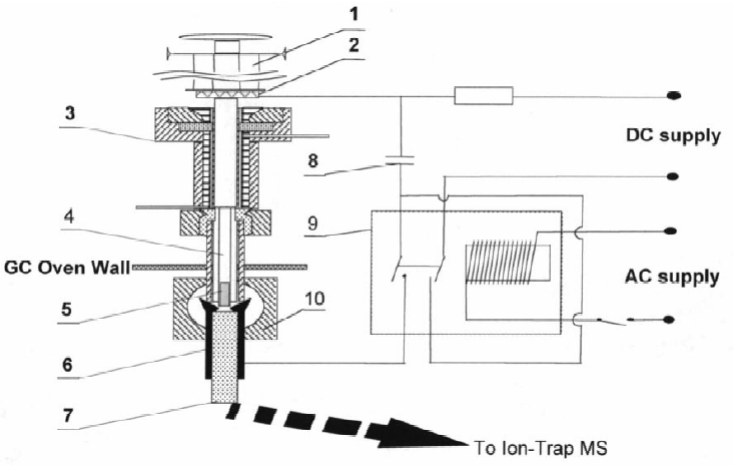
a) Schematic diagram of the flash SPME injector: 1, injector body; 2, washer; 3, septum; 4, nut; 5, needle guide; 6, 0.53 mm I.D. fused-silica capillary; 7, nut; 8, ferrule; 9, heater; 10, butt connector; 11, relay; 12, capacitor; 13, switch.



b) Internally heated SPME device



c) flash desorption injector



Direct capacitive discharge desorption system:
1, SPME syringe;
2, electric connection I;
3, injector body;
4, steel wire;
5, gold coating;
6, electric connection II;
7, transfer line;
8, capacitor;
9, relay;
10, butt connector.

Figure 33. Schematic diagram of GC-SPME injectors

One of the highly sophisticated, fully developed and most commonly used SPME-compatible autosamplers for rapid desorbing the fiber into the GC injection was introduced by CTC Analytics CombiPAL (Analytics, Zwingen, Switzerland) in 1999 [90]. This is a robotic system with a great deal of flexibility for programming SPME analyses (refer to Fig. 34). Samples are loaded onto trays accommodating different vial sizes, and samples are heated and agitated in a

separate sample preparation chamber during the incubation and extraction processes. To facilitate agitation, the sample preparation chamber or agitator tray is rotated at a programmable rotation speed during extraction. The fiber conditioning station is also included in this package, thus allowing “bake-out” or fiber cleaning procedures to be performed after each extraction/desorption cycle outside of the injection port.

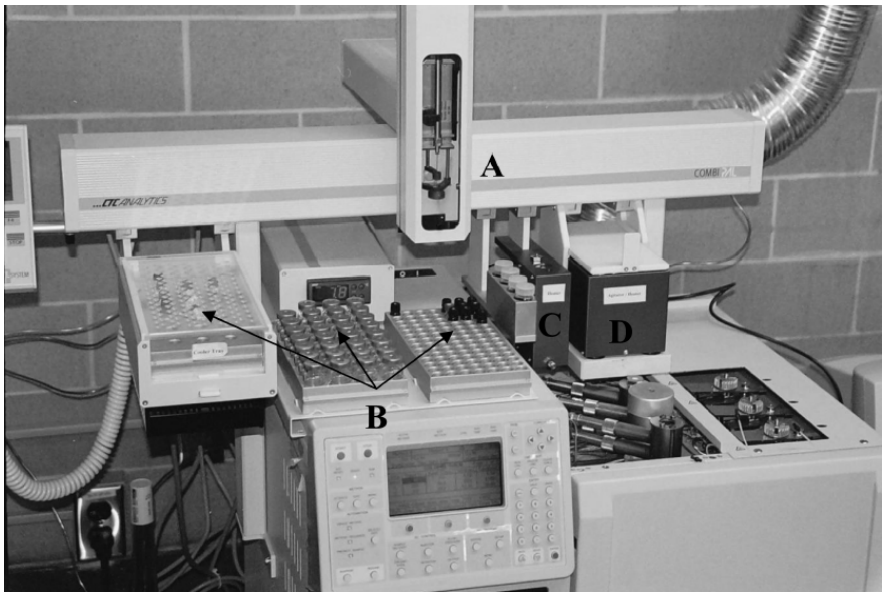


Figure 34. Commercial SPME-GC autosampler (CTC Analytics CombiPAL); A:sample preparation/injection arm, B:sample trays, C:needle heater, D:heater/agitator.

CTC expanded the application range of its GC Injector System CombiPAL introducing the dynamic headspace sampling method so-called *in-tube extraction (ITEX)* where the solvent material is packed within the syringe needle. The enrichment of volatiles can be fully automated as the ITEX device can be mounted on a CTC CombiPAL sampler. The principle of ITEX is illustrated in Fig. 35.

Analytical steps of CombiPAL ITEX cycle are detailed in the following:

i. Sample is heated and/or agitated in a sealed sample vial until equilibrium is achieved.
ii. The trap needle pierces the vial septa and the syringe pumps the headspace through the trap.
iii. The loaded ITEX trap is flash heated up to 350°C and desorbed into the hot GC injector.
iv. After thermal desorption the hot ITEX trap is cleaned with inert flush gas.

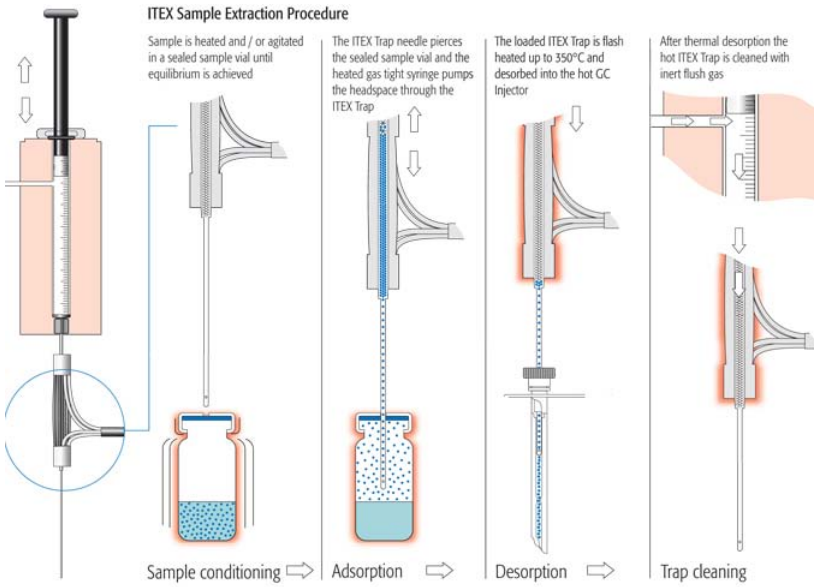


Figure 35. Principle and operation of in-tube extraction (ITEX).

2.1.3.3.2 SPME-HPLC

In this coupling, a fiber SPME might be incorporated with HPLC through a desorption chamber and a six-port injection valve (Fig. 36). The desorption chamber is placed in the position at which the injection loop normally resides on the injection valve. When the injection valve is in the "load" position, it allows the fiber to be introduced into the desorption chamber under ambient pressure. It also allows for the introduction of a desorption solvent if different from the mobile phase. The valve is then switched to "inject" to transfer the desorbed analytes to the HPLC.

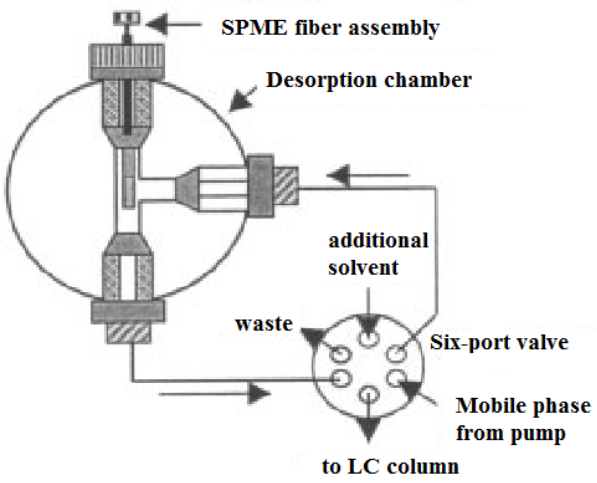


Figure 36. Solvent desorption using for SPME-HPLC interface [93]

2.1.3.3.3 In-tube SPME capillary-HPLC

In-tube SPME capillary using an open-tubular capillary column as the SPME device was developed for coupling SPME with HPLC or HPLC/MS (Fig. 37). The capillary column is generally placed in between the HPLC autosampler needle and the injection valve or inserted in the injection loop of a rotary valve (see Fig.31c). With the in-tube SPME technique, organic compounds in aqueous samples are automatically extracted from the sample into the internally coated stationary phase and then desorbed by introducing a moving stream of HPLC mobile phase or static desorption solvent when the analytes are more strongly adsorbed to the capillary coating [91,94,95].

Fig. 38 illustrates the system based on a modified Spark Holland micro LC autosampler. In this system the analytes are extracted first into the coating by passing sample through the tubing, followed by desorption of the compounds using a small volume of solvent. These approaches, which are suitable for analysis of very small samples (a maximum of few milliliters), also offer convenient interfacing to micro HPLC instrumentation [89,91,96].

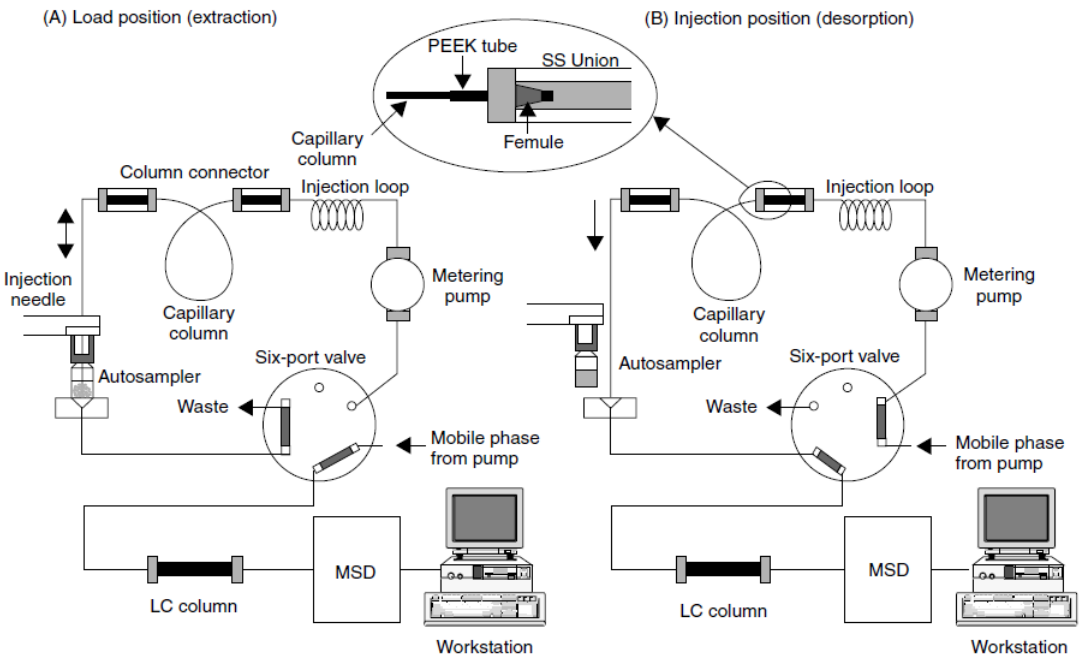


Figure 37. Schematic diagram of automatic in-tube SPME-LC-MS with column switching [91].

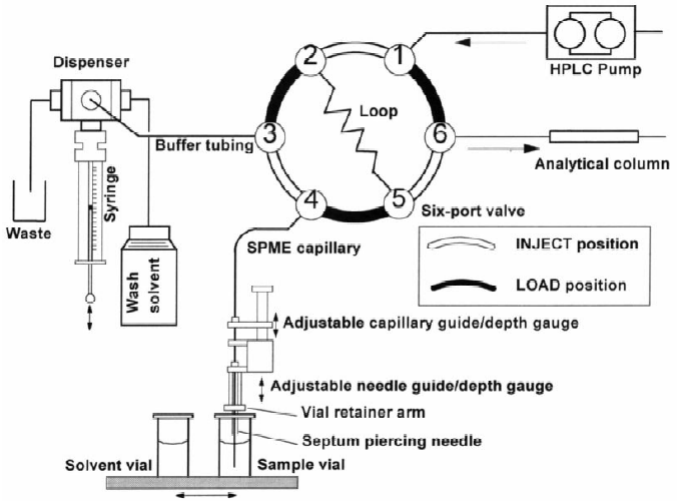


Figure 38. SPME with modified needle to allow automatic in-tube capillary extraction (ITEX)

2.1.3.3.4 Automated SPME in a 96-well plate

Pawliszyn and coworkers [90] invested efforts lately towards automating SPME in a 96-well plate format, which involves the construction of a multi-fiber SPME top plate as shown schematically in Fig. 39. Each of the fibers present on the "brush-like" top plate is aligned to the corresponding well of a commercially available multi-well plate. Agitation methods such as magnetic stirring, mechanical shaking and sonication can be used to reduce the time required for analytes to reach a state of equilibrium between the well contents and the SPME fibers. It is important for the fibers to be able to withstand the agitation conditions used. Preliminary research using SPME in a 96-well plate involved the evaluation of PDMS-coated superelastic wires [97].

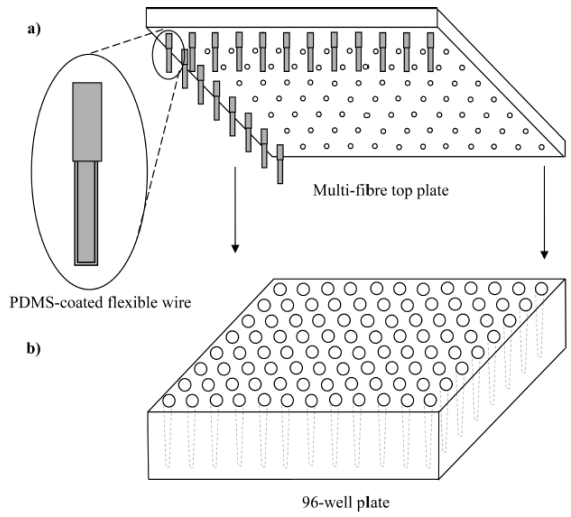


Figure 39. A schematic representation of the multi-fibre top plate used to automate SPME and desorption on a 96-well plate format. (a) For simplification, one row and one column of SPME fibers are shown to be inserted into the top plate. Fibers are constructed from PDMS-coated flexible wire, which is glued by high temperature epoxy resin into a section of 24 gauge needle. This construction can be press-fit into a Teflon block with holes of similar diameter. (b) The multi-fiber top plate can then be placed into commercial multi-well plates for extraction and desorption.

2.1.4 Stir bar sorptive extraction (SBSE)

The stir bar consists of a magnet encased in a glass sheath (Fig. 40). The glass is usually coated with PDMS sorbent (varies from 0.3 to 1 mm) and immersed in the sample to extract the analyte from solution. SBSE uses 50 to 250 times more sorbent than SPME [98-100]. To date, reported SBSE procedures were not usually operated as exhaustive extraction procedures; however, SBSE has a greater capacity for quantitative extraction than SPME. With a larger stir bar, more PDMS coating is deposited, and consequently, a larger sample volume can be extracted.

Extraction of aqueous samples occurs during stirring at a specified speed for a predefined time, depending on the sample volume and the stirring speed, to approach equilibrium. After a given stirring time, the bar is removed from the sample and analytes are eluted with an appropriate solvent or alternatively thermally desorbed using dedicated units (see Fig. 41). In the latter case, the analytes should be cryofocused on a precolumn. Subsequent flash heating transfers analytes into the gas chromatograph. After desorption, the stir bar is expected to be reused.

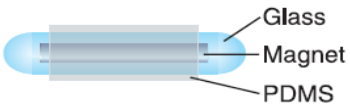


Figure 40. Schematic representation of a stir bar resorted to SBSE.

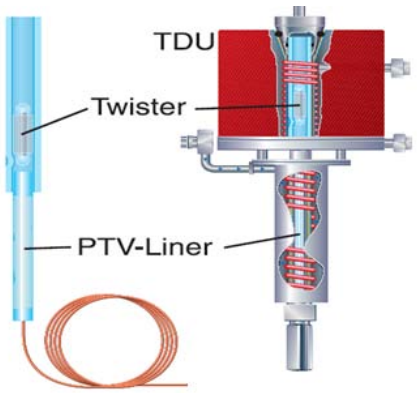


Figure 41. Schematic representation of the desorption unit for SBSE.

SBSE has been proven suitable for trace enrichment of organic compounds from aqueous food, biological, and environmental samples [98-102]. Extraction remains a balancing act between sorbent mass and sample volume, and it appears that the primary advantage of SBSE using the PDMS sorbent (i.e., greater concentration capability than SPME) will also be its

greatest disadvantage. The nonselective sorptive capability of the PDMS sorbent co-concentrates undesirable matrix components from solution. Thus, SBSE produces analyte accumulation in the sorbent but not sample cleanup. Nevertheless, SBSE alike SPME is attractive because it might operate as a solventless enrichment technique. That coupled with the rapidity and ease of use of this procedure makes it a desirable microextraction approach for analysts.

2.1.5 Microextraction by packed sorbent (MEPS)

Microextraction by packed sorbent (MEPS) is a new development in the fields of sample preparation and sample handling that uses a gas tight syringe as extraction device. MEPS is the miniaturization of conventional SPE packed bed devices from milliliter bed volumes to microliter volumes. In MEPS the sorbent material, about 1 mg, is either inserted into the syringe barrel as a plug with polyethylene fibers on both sides, or between the syringe barrel and the needle (Fig. 42). The MEPS approach to sample preparation is suitable for reversed phases, normal phases, mixed mode or ion exchange chemistries. MEPS is currently available in a variety of common SPE phases [92,103].

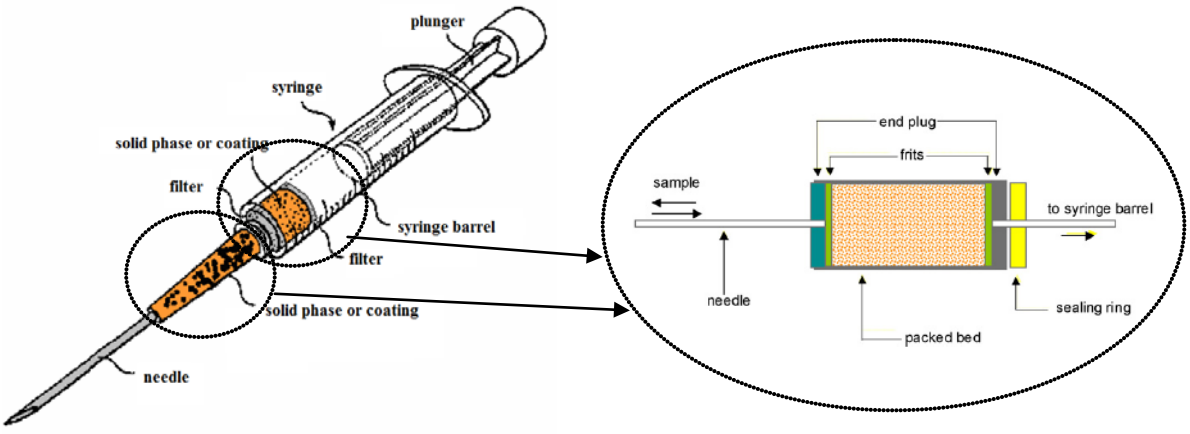


Figure 42. Schematic picture of microextraction by packed sorbent (MEPS)

The sample processing steps in MEPS (see Fig. 43) are similar to those of SPE. Basically, after conditioning of the sorbent with an appropriate solvent, the sample solution is drawn through the needle into the syringe by an autosampler (which pumps the sample up and down once or several times) for uptake of analytes. This is followed by a washing step to remove interferences and then eluted analytes of interest with an organic solvent, such as methanol or the LC mobile phase itself, are directed to LC or thermally desorbed prior to GC assays.

MEPS can be connected to LC or GC without any modification of the instrument. A variety of sorbent materials including reversed phase silica based (C2, C8, C18), restricted access material (RAM) or molecular imprinted polymers (MIPs) have been used to date [103-106].

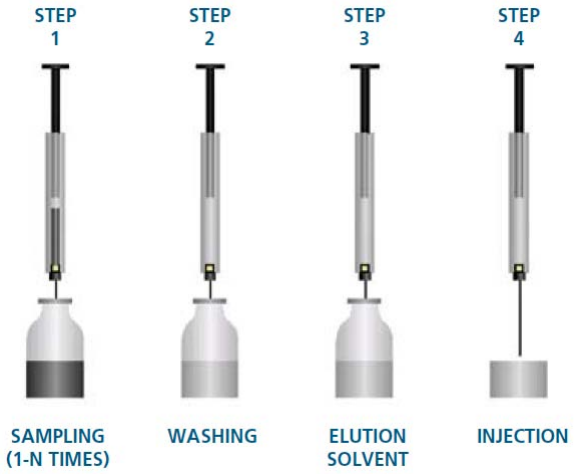


Figure 43. A scheme of MEPS (the process is fully automated).

2.1.6 Comparison of microextraction techniques

In the review by Chen et al. [16], sample preparation techniques (e.g., LLE, SPE, SPME, and SBSE) were critically evaluated. When examined collectively, sample processing techniques can be perceived as variations of a single principle as practiced by today's analysts (Fig. 44). Two fundamentals drive extraction procedures: (i) determining the value of diffusion constant, K_d, for a given analyte-sample matrix-sorbent combination, which will indicate if the process is an equilibrium procedure (in nonequilibrium procedures, K_d approaches infinity during sorption), and (ii) determining if the majority of the analyte (>90%) is recovered from the sample (Table 1), which will indicate if the process used is exhaustive. K_d is the continuum that relates the procedures discussed. As commonly implemented, K_d values for the studied procedures decrease in the order $K_{d(SPE)} > K_{d(LLE)} \approx K_{d(SBSE)} > K_{d(SPME)}$. As commonly practiced, SPE and SPME exist at opposite ends of the continuum in method fundamentals. LLE is an equilibrium procedure, but through application of repeated extractions, nearly quantitative, or exhaustive, recovery of analytes can be achieved. SBSE is a recently emerging procedure that appears to lie on the extraction continuum between LLE and SPME. The capacity of SBSE for exhaustive extraction is greater than SPME but less than LLE. The capacity for quantitative, or exhaustive, transfer is related to the K_d value and the total mass of sorbent utilized.

Compared to nonequilibrium methods, equilibrium methods tend to be simpler, and more selective, therefore require less cleanup but require determination of pre-equilibrium/ equilibrium status, are time, temperature, and matrix dependent, and require internal standards for calibration [46,107]. Extraction approaches differ, but the choice of methodology depends on the analyst's objectives and resources and the analytical properties to be optimized.

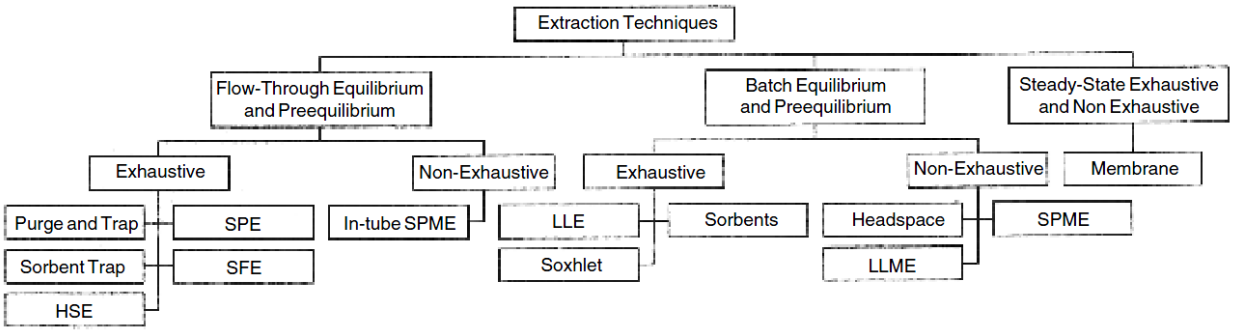


Figure 44. Classification of sample preparation techniques.

Table 1 Extraction method fundamentals

technique	K_d	%recovery
SPE	Non-equilibrium	Exhaustive
MEPS	Equilibrium	Non-exhaustive
LLE	Equilibrium	Exhaustive
SBSE	Equilibrium	Non-exhaustive
SPME	Equilibrium	Non-exhaustive

2.2 Sample preparation techniques for metal fractionation and/or speciation in solid samples

2.2.1 Extraction/leaching tests

Extraction is the process whereby a mixture of several substances in the liquid phase is at least partially separated upon addition of a liquid solvent in which the original substances have different solubility. When some of the original substances are solids, the process is called *leaching*. In general, a leaching test involves contacting a solid material with a leachant to determine which components in the solid will dissolve in the leachant and create a leaching solution or leachate. To investigate the various processes governing the extent and rate of leaching, endless variations can be introduced by changing test variables, such as leachant composition, method of contact, liquid-to-solid (L/S) ratio, contact time and system control (e.g., pH, temperature).

Leaching tests have a wide range of objectives, the most common of which are: (i) characterizations of pollution sources, (ii) evaluation of element mobility and bioaccessibility, and (iii) identification of binding site of elements for assessing accumulation, pollutant and transport mechanisms of elements. The leaching behaviour of a particular constituent in a material is not related to the total amount of that component in the material. Since uptake by organisms and transport into the environment is largely controlled by release in the water phase, knowledge regarding the leaching/extraction behaviour of pollutants is crucial in order to assess environmental release or environmental impact.

In all fields, three main levels of aggressiveness in testing can be distinguished following total composition (total destruction), a potentially leachable (bioaccessible) amount, (using relatively aggressive agents) and actual leachability or bioaccessibility under normal exposure conditions (using a mild method of extraction).

For the purposes of this discussion, leaching tests have been separated into two broad categories on the basis of whether or not the leachant is renewed: (i) static leaching tests (without leachant renewal), and (ii) dynamic leaching tests (with leachant renewal).

2.2.1.1 Static leaching tests

Static leaching tests include all tests in which a specific quantity of leachant is contacted with a specific quantity of solid for a certain length of time, without leachant renewal. The leachate is separated from the solid and analysed either at various times during the test, or, as in most extraction tests, at the end of the test. The analysis of leachates generated at various times can help determine the kinetics of the leaching process. The underlying assumption in this type of test is that an equilibrium condition is achieved by the end of the extraction test, that is, the concentrations of solutes in the leachate become constant. In this no-flow system, an equilibrium condition occurs when there is no net transfer of components from the solid phase to the leaching solution.

Static leaching tests can be further divided into four subcategories: (i) agitated single leaching tests, (ii) non-agitated leaching tests, (iii) sequential extraction tests, and (iv) concentration buildup tests.

2.2.1.1.1 Agitated single leaching tests

Agitated leaching tests are performed to reach steady-state conditions as quickly as possible. They measure the chemical properties of a solid-leachant system regardless of the original particle size, as opposed to tests based on rate-limiting mass transfer mechanisms. Agitation ensures a homogeneous mixture, promotes contact between the solid and the leachant and reduces boundary layer thicknesses. Sample particle size reduction is often performed to increase the surface area to volume ratio of the solid to enhance liquid/solid phase contact and to eliminate mass-transfer limitations (see Fig. 45).

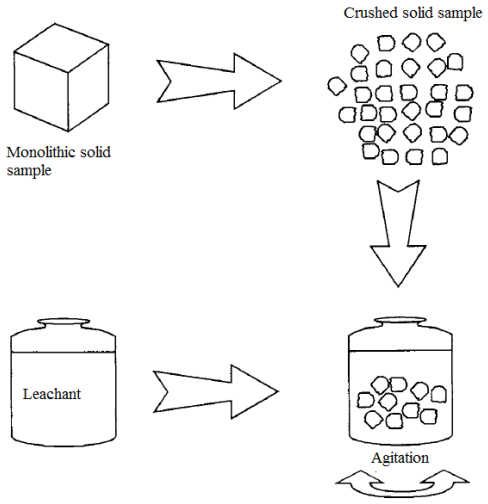


Figure 45. Agitated leaching tests

Generally, this reduces the duration of the test by reducing the time required to reach a pseudo-equilibrium condition in the leachate. This procedure may also have the effect of overestimating the short-term release of constituents. A steady-state leaching environment can also be attained in a column apparatus by recirculating the collected leachate back into the column.

Amongst the various agitated batch (single) extraction tests that are used for regulatory purposes or for research the toxicity characteristic leaching procedure (TCLP), and the extraction procedure toxicity (EP TOX) are worth mentioning. All the methods specify the type of leaching vessel to be used, the type of sample preparation that is required, the amount of sample that is needed, the type of leachant to be employed, the L/S ratio that is used, the type of agitation that is required and the duration of the test. Most methods also specify the type of filtration that is to be employed to allow for quantification of total dissolved

constituents in the leachate [108]. TCLP has been applied for characterization of fly ash disposal in this thesis [109] so that TCLP test is briefly discussed in following paragraph. A solid waste material has the characteristic of “toxicity” if it is able to leach specific toxic metals, organic compounds, or pesticides into the soil or groundwater under landfill conditions.

The toxicity characteristic leaching procedure (TCLP), USEPA Method 1311, is the United Stated Environmental Protection Agency (USEPA) method required to determine whether a solid waste is a hazardous waste [110]. The TCLP test is a single extraction scheme that simulates conditions of wastes if they were to be disposed of in an ordinary sanitary landfill. Waste samples vary widely and may be completely solid or completely liquid, or contain both liquids and solids, such as sludge samples. If a waste contains less than 0.5% solids, only the filtered liquid needs to be tested and the leaching procedure does not need to be performed. A sample composed of both liquid and solid materials must be filtered to separate any liquid. The liquid portion is saved for further testing. The remaining solid sample (particle size <9.5mm) is then mixed with a slightly acidic extraction liquid equal to 20 times the weight of the original sample being tested. Two leachants are specified in the procedure and the leachant employed is a function of the pH of the above leachate. For moderate to highly alkaline wastes a pH 2.88 (0.1M acetic acid solution) is used, while other wastes are leached with a pH 4.93 using a 0.1M acetic acid/acetate buffer solution.

The extraction procedure lasts 18 hours and is carried out by tumbling a zero headspace bottle containing the solid and liquid in an end-over-end fashion at 30±2 rpm. This tumbling simulates the leaching action of water seeping through waste in a landfill. After tumbling, the sample/extraction fluid mixture is filtered to separate the solid material from the extraction fluid. The solid portion is discarded. The remaining extraction fluid is then combined with any liquid filtered from the original sample. The combined liquid sample is then analyzed using specific analytical procedures for various metals and chemical compounds. The concentrations of each constituent are reported, usually in milligrams per liter (mg/L). If the concentration of that constituent is greater than the endorsed TCLP limit, the waste is regarded as a “toxicity characteristic” hazardous waste.

2.2.1.1.2 Non-agitated leaching tests

A non-agitated leaching test is performed to study the physical mechanisms that are rate limiting in leaching. The underlying assumption behind a non-agitated leaching test is that the physical integrity of the solid matrix and mass transfer constraints (both internally within the sample and externally in the boundary layer) affect the amount of contaminants that are leached during the test. They can be performed on large particle-sized solid samples, concrete-type or monolithic samples (see Fig. 46).

The disadvantage of running a non-agitated leaching test is that a much longer contact period may be required to reach equilibrium conditions than is required in an agitated leaching test. The advantage of this type of test is that rate-limiting mechanisms of leaching due to the physical integrity of the solid matrix are taken into account.

a) Static test with monolithic solid sample

b) Static test with non-monolithic large solid particles

Figure 46. Static leaching tests

2.2.1.1.3 Sequential extraction tests

A sequential chemical extraction test is composed of a battery of agitated extraction tests (Fig. 47). It involves performing sequential elutions of a given sample aliquot with different leachants, that is, A, B, C, D and E in Fig. 47, which are increasingly more aggressive in terms of chemical attack toward the residue. The amount extracted in each elution is associated with a certain chemical form or mineral phase in the solid phase. Within this category, we find the overall leaching tests compiled in table 2 (see page 64).

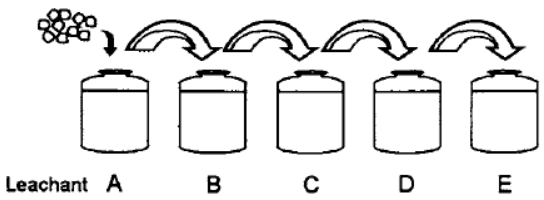


Figure 47. Sequential extraction tests

2.2.1.1.4 Concentration buildup tests

In a concentration buildup test, extraction is achieved at a very low cumulative L/S ratio. Aliquots of samples are successively contacted with the same leachant (Fig. 48). The contact of leachate with fresh solid material can be considered as a model for an elemental volume of water flowing through a large body of solid or sediment and approaching saturation with respect to specific mineral phases. The purpose of this test is not to collect kinetic information, but to characterize a leachate saturated with soluble constituents from the solid materials. In some cases, this may simulate the actual pore water composition of a granular material in column leach tests or in outdoor disposal or utilization scenarios.

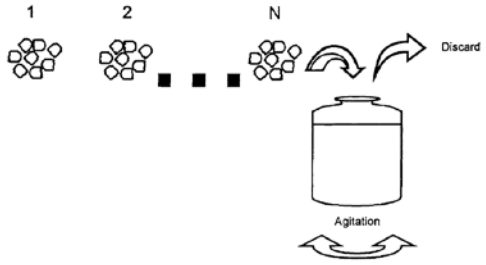


Figure 48. Concentration buildup tests

2.2.1.2 Dynamic leaching tests

Dynamic tests include all tests in which the leachant is continuously or intermittently renewed to maintain a driving force for leaching and thus insight is obtained into the release of metals under worst case scenarios. The intermittent tests may be conducted by alternating leaching periods with dry periods to study the effects of desiccation or unsaturated flow conditions. Dynamic tests provide information about the kinetics of solid phase dissolution and contaminant flux. Information is generated as a function of time, and attempts are often made to preserve the solid's physical integrity. These two factors lend this category of leaching tests to the investigation of more complex mechanisms of leaching.

Dynamic leaching tests can be further divided into subcategories according to how the interface between the solid and the leachant is defined. Leaching tests in which individual solid particles are used to define the interface are called *serial batch leaching tests*. The leaching tests in which a characteristic dimension of the solid (such as the external geometric surface area or the geometric surface area perpendicular to flow) is used to define the interface include *flow-around leaching tests* and *flow-through or column leaching tests*.

2.2.1.2.1 Serial batch leaching tests

A serial batch leaching test is conducted using a granular or crushed sample which is mixed with leachant at a given L/S ratio for a specified period of time (Fig. 49). The leachate is then separated from the solids and replaced with fresh leachant until the desired number of leaching periods have been completed. The solid/leachant mixture is normally agitated to promote contact. Kinetic information regarding contaminant dissolution is obtained using the concentrations measured in the leachate from each of the leaching periods. Data from serial batch leaching tests can be used to construct an extraction profile or extractogram to infer the temporal release of leachable constituents.

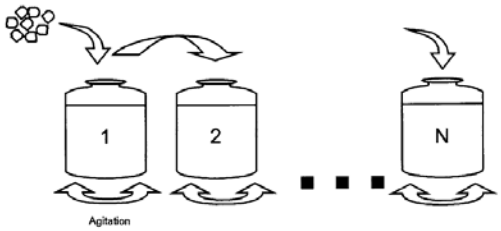


Figure 49. Serial batch tests

2.2.1.2.2 Flow-around leaching tests

In flow-around tests, a solid sample is placed in the leaching vessel and the flow of fresh leachant around the sample provides the driving force to maintain leaching. The L/S ratio is modified to express the volume of leachant divided by the surface area of the solid sample. Samples are usually monolithic, although non-monolithic or crushed solid residue may be used. Agitation is generally not performed. Leachant flow is either continuous (Fig. 50a), in which case it is sampled and analysed periodically, or it is intermittently renewed (Fig. 50b). The latter method is generally simpler from an experimental point of view, but the renewal frequency must be sufficient to prevent a buildup of contaminants at the solid/leachant interface, which may inhibit further leaching by reducing the diffusional gradient.

a) continuous leachate renewal b) intermittent leachate renewal

Figure 50. Flow-around leaching tests

2.2.1.2.3 Flow-through leaching tests

In a flow-through or column test, an open container is packed with a porous solid and leachant is passed through, either continuously or intermittently. The effluent is sampled periodically and analysed for the parameters of interest. The results are used to examine contaminant removal in which the primary transport mechanism is advection. There are two basic types of flow-through tests characterized primarily by the shape and size of the container. The first type is a column test which is performed using a small cylindrical container (Fig. 51a). The second is a lysimeter test which is conducted in a large rectangular or cylindrical container (Fig. 51b). In general, the size of the sample used in a flow-through leaching test tends to be large to minimise the effects of sample heterogeneity and wall channelling effects. Columns may be operated either in an upflow or downflow mode, whereas lysimeters are always operated in a downflow mode. The flow of leachant through the solid depends upon its hydraulic conductivity, as well as the hydraulic gradient, and varies with the individual leaching test.

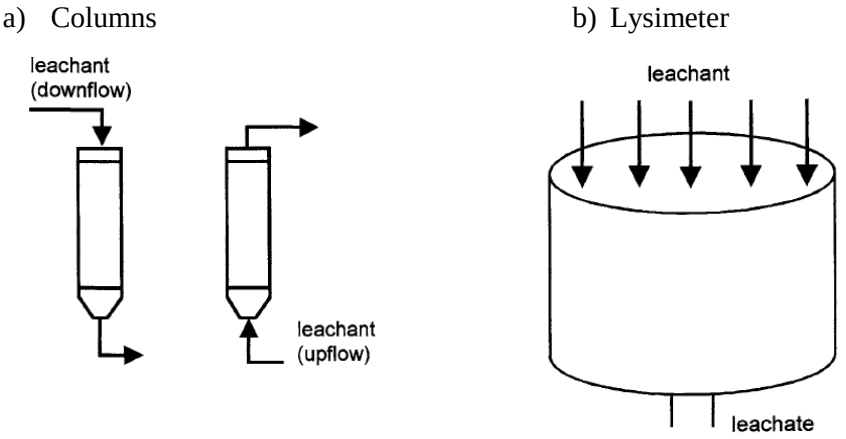


Figure 51. Flow-through leaching tests

Minicolumns may be used to achieve a relatively rapid breakthrough of leached species (exhaustive extraction). Since head losses may be large and a rapid breakthrough is desired, the leachant is usually delivered under pressure and at a constant flow rate. The advantages of minicolumns include: (i) L/S ratios that are similar to those of real solid-leachant systems, (ii) a known and easily varied average fluid velocity, (iii) negligible axial/radial dispersion or spreading of the solute, (iv) a simple estimation of both equilibrium and kinetic coefficients, and (v) automation permitting the rapid output of data. These tests are not applicable when large volumes of leachate are needed because of undue dilution of analytes. Care should also

be taken when conducting flow-through leaching tests to avoid unnatural channelling of water and clogging by fine material or biological growth. In lysimeter leaching tests, channelling cannot be avoided. It is a factor that occurs in the field, and its influence should be modeled in the laboratory, although quantifying it is difficult. Biodegradation of organics can also be a problem in columns, although in some cases experiments are intentionally set up to measure the effects of biological activity. Flow-through leaching tests can also be modified to examine other site-specific influences, such as vegetation on the surface of the container, or layered media, such as ash and geological material.

2.2.2 Speciation and fractionation of trace elements

Trace elements play an important role in the functioning of life in ecosystems. Some elements can be highly toxic to various life forms; others are considered essential, but can become toxic at higher doses. Many of these effects depend strongly on the particular form in which the element is present in the system. For example, Cr(VI) ions are considered far more toxic than Cr(III). On the other hand, while both methylmercury and inorganic mercury are toxic, they show different patterns of toxicity. Often these different chemical forms of a particular element or its compounds are referred to as "species". The notion that the distribution among its various species will have a major effect on the behavior of a particular element has been accepted in such diverse fields as toxicology, clinical chemistry, geochemistry, and environmental chemistry. New developments in analytical instrumentation and methodology now often allow us to identify and measure the species present in a particular system. Because of these possibilities, numerous publications have appeared in which the term "speciation" is employed. However, this term has been used in a number of different ways, including the transformation of species, the distribution of species, or the analytical activity to determine the concentrations of species.

The International Union for Pure and Applied Chemistry (IUPAC) has defined chemical species and elemental speciation in chemistry as follows [111]:

i. *Chemical species*. Specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure.

ii. *Speciation analysis*. Analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample.

iii. *Speciation of an element.* Distribution of an element amongst defined chemical species in a system.

When elemental speciation is not feasible, the term fractionation is in use, which is defined as follows:

iv. *Fractionation*. Process of classification of an analyte or a group of analytes from a certain sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties.

An assessment of the impact of an element cannot be made based only on its total concentration. It is, however, often not possible to determine the concentrations of the different chemical species that sum up to the total concentration of an element in a given matrix. Often, chemical species present in a given sample are not stable enough to be determined as such. During the measurement process the partitioning of the element among its species may be changed. This behavior can be caused by, for example, a change in pH necessitated by the analytical procedure, or by intrinsic properties of measurement methods that affect the equilibrium between species. In some cases, species are stable enough to be determined as such (e.g., tetraethyllead, arsenobetaine, cyanocobalamin). In a given situation the determination of the concentration of such a species may be what is actually desired. However, this does not mean that the speciation of the element has been determined, only the concentration of one or a few species. The direct determination of labile species at their natural levels requires noninvasive methods such as direct spectroscopic or potentiometric measurements that do not perturb the sample. In many cases the large numbers of individual species (e.g., in metal-humic acid complexes or metal complexes in biological fluids) will make it impossible to determine the speciation. The practice has been to identify various classes of species of an element, that is, fractionation, and to determine the sum of its concentrations in each class. Fractionations can be based on many different properties of the chemical species, such as size, solubility in given chemicals, charge, and hydrophobicity. Fractionation may involve an actual physical separation (e.g., filtration, size-exclusion chromatography). In some instances, fractionation may be refined by supplementary speciation analysis. For example, for copper in natural waters a fractionation can be performed to determine organic and inorganic copper concentrations. With further analyses and calculations the inorganic fraction can be subdivided into individual species. It is also desirable to measure the total concentration of the element in order to verify the mass balance [109,112].

2.2.3 Bioavailability and bioaccessibility

Many definitions are found in the literature when referring to bioavailability and bioaccessibility of pollutants which create confusion for environmental scientists, and this ambiguity is undesirable given current regulatory contexts. Therefore, the U.S National Research Council (NRC) reported the definitions of *bioavailability* and *bioaccessibility* [113], as detailed below. The ISO/TS 17924 norm [114] entitled *Soil quality-Assessment of human exposure from ingestion of soil and soil material* makes also a clear difference between these two terms.

Bioavailable. This term has an implied immediacy what is available is available now. Hence, NRC define a *bioavailable compound* as that which is freely available to cross an organism's cellular membrane from the medium the organism inhabits at a given time (Fig. 52). Once transfer across the membrane has occurred, storage, transformation, assimilation, or degradation can take place within the organism, however, these processes are obviously distinct from the transfer between the medium (e.g., soil) and the organism.

Bioaccessible. The definition implies that some of what is accessible can be reached but is often not quite within reach from a given place or at a given time. In this context, a constraint is implied in time and/or space, preventing the organism from gaining access to the chemical now. Hence, NRC define the *bioaccessible compound* as that which is available to cross an organism's cellular membrane from the environment if the organism has access to the chemical, and most often refers in environmental solids to contaminants released in the liquid phase. It should be born in mind that the chemical may be either physically removed from the organism or only bioavailable after a period of time. In this context, *physically removed* may refer to a chemical that is occluded in soil organic matter and hence is not bioavailable (although bioaccessible) at a given time or that occupies a different spatial range of the environment than the organism (Fig. 52). Contaminants can become available within the order of seconds from these locations (and hence are bioavailable), following release from labile or reversible pools; or, the organism can move into contact with the contaminant. Alternatively, release may occur over much longer timescales (e.g., years or decades) and render the chemical bioaccessible. To sum up, bioaccessibility encompasses what is actually bioavailable now plus what is potentially bioavailable. Bioaccessible fractions are those to be determined with chemical fractionation methods (see below) and as the definition implies assays with biota are not needed.

Figure 53 describes the bioavailability and bioaccessible processes (**A-D**). **A** refers to the physical, chemical, and biochemical phenomena that bind, unbind, expose, or solubilize a contaminant associated with soil or sediment. Contaminant-solid binding may occur by adsorption on solid surfaces, by absorption within a phaselike natural organic matter, or by precipitation, whereas contaminant release to fluids in contact with the soil or sediment occurs in response to changes in water saturation, in water and gas chemistry, and in solid surface properties. Time is an important aspect governing contaminant-solid interactions. Contaminants are transformed or incorporated into more stable solid phases over time, which can lead to a decrease in contaminant bioaccessibility-a process referred to as aging. **B** involves the movement of a released (bioaccessible) contaminant to the membrane of an organism, whereas **C** involves the movement of contaminants still bound to the solid phase.

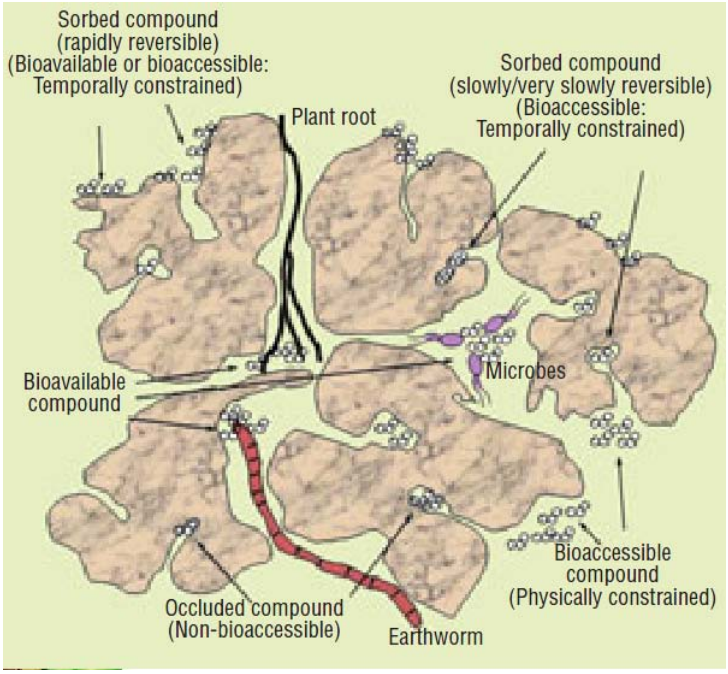


Figure 52. Diagram illustrating the bioavailable and bioaccessible fractions of a contaminant in soil as defined by physical location. It also describes the relationship of soil-associated contaminant molecules in relation to bioaccessible fraction [115].

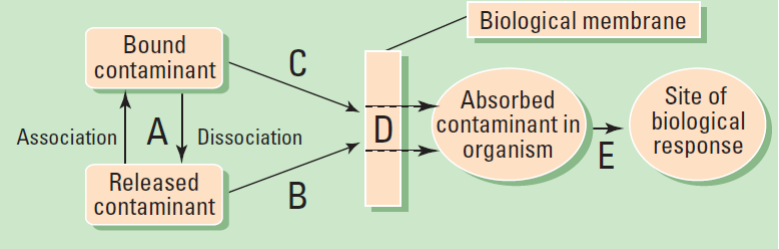


Figure 53. In both soil and sediment, processes that determine exposure to contamination include release of a solid-bound contaminant (A) and subsequent transport (B), transport of bound contaminants (C), uptake across a physiological membrane (D), and incorporation into a living system (E). Note that A, B, and C can occur internal to an organism, such as in the lumen of the gut [113].

D entails movement from the external environment through a physiological barrier and into a living system corresponding to the bioavailable fraction. Because of the enormous diversity of organisms, the actual process of contaminant uptake into a cell or factors that may impede or facilitate uptake-varies depending on receptor type. Human uptake mechanisms include absorption across the gut wall, the skin, and the lining of the lungs. One common factor among all organisms is the presence of a cellular membrane that separates the cell interior from the external environment, through which most contaminants must pass before deleterious effects on the cell or organism occur. **E** refers to paths taken by the chemical following uptake across a membrane, for example, metabolic processing or exerting a toxic effect within a particular tissue. Of particular importance is the bioaccumulation of contaminants within tissues that are often inaccessible to normal elimination mechanisms, such as metabolism and excretion. Slow release of the chemicals from these storage sites can cause protracted "exposure" within the body even when the external exposure has been reduced. In addition, bioaccumulated contaminants (e.g., polychlorinated biphenyls) may become available at some point to higher order organisms that eat the plant or animal in which the contaminants are stored.

Different trace elements are widespread in the environment as a result of natural processes and human activities (agriculture, industry, and transportation). Many compounds can accumulate in environmental solid substrates and can causes serious problems for living organisms. Therefore, ascertainment of the contamination of solids has attracted special attention as point of reference for ecology and environmental management. At present, it is widely recognized that the distribution, mobility, toxicity and bioavailability of heavy metals and radionuclide in the environment depends not only on their total concentration but also on the association form in the solid phase to which they are bound. Some variations of the chemical or physical

conditions in the environment can accelerate to some extent the release of toxic metals into it, thus causing bioaccessible contaminants. Indeed, data on total content of elements are quite insufficient to estimate the possible risk of remobilization of trace element under changing environmental conditions. Thus, procedures for distinguishing the phase association and binding sites of elements in soils, sediments, and solid wastes are required. Bioaccessibility of hazardous metals depends greatly on the characteristics of the particle surface, on the kind of strength of the bond and on the properties of the solution in contact with the solid samples.

A widely used technique for understanding element distribution in the solid phases and thus determining bioaccessible forms is based on the application of sequential chemical extractions for metal fractionation [116-123]. These methods are based on the rational use of a series of more or less selective reagents, chosen to solubilise successively the different mineralogical fractions, thought to be responsible of natural and/or anthropogenic modifications of environmental conditions. Sequential extraction schemes make use of suitable reagents which are applied in a given order to the sample, the number of stages and the choice of the specific reagent used in each one, being dependent of the goals pursued and on the physical characteristics of the target sample. The choice of the type of extractant should be strictly correlated with: (i) the nature of target species, (ii) the chemical forms to be released, (iii) the matrix from which the compounds are to be extracted, and (iv) the analytical techniques available in the laboratory for the final determination. A summary of extraction schemes for metal species is shown in Table 2. Among them, the most widely used sequential extraction schemes are those proposed by Tessier *et al.* [124] and the Standards, Measurements and Testing (SM&T) Program of the European Commission (formerly the Commission of the European Communities Bureau) [125]. These schemes have been demonstrated to give satisfactory results for the targeted phases owing to a careful selection of reagents and operating conditions. In all schemes, extractants are applied in order of increasing reactivity so that the successive (bioaccessible) fractions obtained correspond to metal association forms with lesser mobility (Fig. 54). The extractants more commonly used in sequential extraction schemes for partitioning of metals fall generally within the following groups: unbuffered salts, weak acids, reducing agents, oxidising agents, and strong acids.

While the release of trace elements is important from the point of view of nutrition or ecotoxicity of bioaccesible/bioavailable species, their retention is important in designing specific physico-chemical treatment for binding them to immobile (not bioaccessible)

fractions. Direct determination of speciation in the solid material, without prior separation of the species from the solid matrix, is generally limited to major component elements since few of the direct techniques available are sensitive enough for trace element studies. Resorting to separation or extraction of element species presents the usual problem of maintaining the speciation unchanged during extraction. This explains the widespread acceptance of fractionation tests.

Table 2 Some representative sequential extraction schemes for trace element fractionation

Scheme	Step[a]						
	A	**B**	**C**	**D**	**E**	**F**	**G**
MacLaren and Crawford (1973)	$CaCl_2$	HOAc	-	$K_4P_2O_7$	NH_4Ox/HOx	-	DCB
Gibbs (1977) [126]	$MgCl_2$	-	-	NaOCl/ DCB[C]	-	-	DCB
Engler et al. (1979) [127]	NH_4OAc	-	$NH_2OH \bullet HCl$	-	-	H_2O_2/NH4OAc	DCB
Tessier et al. (1979) [124]	$MgCl_2$	NaOAc	-	-	$NH_2OH \bullet HCl$/ HOAc	H_2O_2/NH4OAc	-
Meguelllati et al. (1983) [128]	$BaCl_2$	NaOAc[C]	-	-	$NH_2OH \bullet HCl$/ HOAc[D]	H_2O_2/NH4OAc[B]	-
Shuman (1983) [129]	$Mg(NO_3)_2$	-	$NH_2OH \bullet HCl$[c]	NaOCl[B]	NH_4Ox/HOx	-	-
Salomons and Förtsner (1984) [130]	NH_4OAc	NaOAc	$NH_2OH \bullet HCl$	-	NH_4Ox/HOx	H_2O_2/NH_4OAc	-
Miller et al. (1986) [131]	$Ca(NO_3)_2$/ $Pb(NO_3)_2$	HOAc/ $Ca(NO_3)_2$	$NH_2OH \bullet HCl$	$K_4P_2O_7$	NH_4Ox/HOx	-	NH_4Ox/HOx
Elliot et al. (1990) [132]	$MgCl_2$	NaOAc	-	$Na_4P_2O_7$[D]	NH_4Ox/HOx[C]	-	-
Ure et al. (1993) (BCR) [133]	-	HOAc	$NH_2OH \bullet HCl$	-	-	H_2O_2/NH_4OAc	-
Krishnamurti et al. (1995[134])	$Mg(NO_3)_2$	NaOAc	$NH_2OH \bullet HCl$[D]	$Na_4P_2O_7$[C]	NH_4Ox[F]	$H_2O_2/Mg(NO_3)_2$[E]	NH_4Ox/AA
Campanella et al. (1995) [135]	-	NH_4OAc	$NH_2OH \bullet HCl$ /HOAc	-	-	HCl[C]/$NaOH$[D]/ HNO_3[E]	-
Sahuquillo et al. (1999) [125] (Modified BCR)[b]	-	HOAc	$NH_2OH \bullet HCl$	-	-	H_2O_2/NH_4OAc	-
Fedotov et al. (2006) [136]	$Ca(NO_3)_2$	HOAc	$NH_2OH \bullet HCl$	$K_4P_2O_7$	NH_4Ox[F]		

[a]When the order of attack differs from that shown in the table, this is indicated by the superscript. Phases; A: exchangeable, B: acid soluble, C: easily reducible (i.e. Mn Oxides), D: easily oxidisable (i.e. humic and fulvic acids), E: moderately reducible (i.e. amorphous Fe oxides), F: oxidisable oxides and sulfides, G: poorly-reducible (i.e. crystalline Fe-oxides). The residual fraction is not included in the table. [b]This scheme differs mainly from that of Ure et al.[133] in the hydroxylamine hydrochloride concentration (0.5 instead of 0.1 mol L^{-1} and the pH of this extractant (1.5 instead of 2). AA: ascorbic acid, OX: oxalate, DCB: dithionite-citrate system buffered with $NaHCO_3$

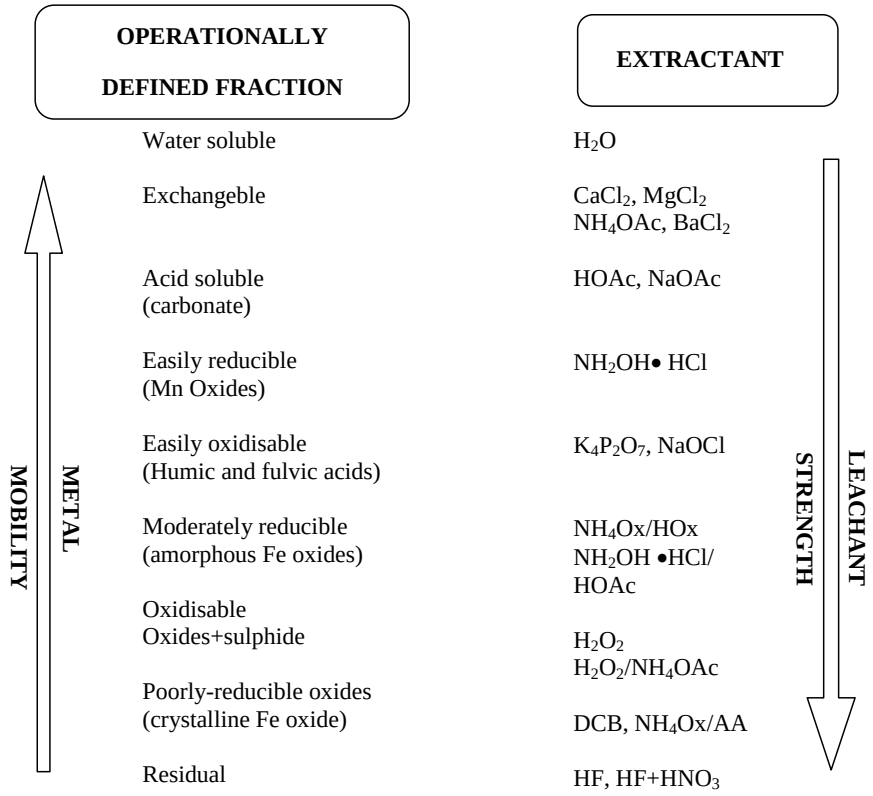


Figure 54. Relationship between metal mobility in the different operationally-defined phases and leachant strength of common chemical reagents used for sequential extraction [117], (AA: ascorbic acid, OX: oxalate, DCB: dithionite-citrate system buffered with $NaHCO_3$).

2.2.4 Targeted phases in environmental solid samples

As indicated above, metal fractionation is achieved by a set of chemical reagents, which are sequentially applied to the solid sample aliquot. Most procedures involve the chemical separation into metal soluble in water and acid medium, exchangeable, reducible (or associated with Fe and Mn oxides), oxidizable (or associated with organic matter and sulfur), and residuals or associated with silicates) or specified in Fig. 54. The relationship between metal fractions obtained with different chemicals and bioaccessibility of associated species is described below.

2.2.4.1 Water-soluble fraction

Trace elements extracted by water are relatively labile and thus readily bioaccessible [137]. The water-soluble fraction of a soil or sediment is the first to be uptaken by biota. This fraction is usually negligible, except in areas where evaporites are present [138]. This phase contains the water-soluble species made up of free ions and ions complexed with soluble organic matter and other constituents.

The use of water as an extractant is connected with analytical and methodological problems: (i) the organic matter content of the sample significantly influences the interaction between the water and the solid phase (e.g. by suspension forming), (ii) the water as an extractant medium has no buffering capacity, therefore the easily solubilized major components of the soil or sediment strongly influence the results of a long-term leaching of trace elements. When this procedure is used the pH may not be calculable because of the low buffering capacity of the extractant and additionally problems with re-adsorption might occur [139], (iii) the efficiency of the extractant for trace elements is too low and therefore the dissolved concentration may be outside the range of determination limits of available routine analytical methods (FAAS, ICP-AES) [140]. The actual water-soluble fraction may also be obtained in-situ by sampling soil or sediment pore solution using dialysis tubes or microsuction cups.

2.2.4.2 Exchangeable fraction

This fraction includes weakly adsorbed metals on the solid surface, *namely*, metals that can be released by ion-exchange processes. Changes in the ionic composition of soil solution, influencing adsorption-desorption reactions, or lowering of pH could cause remobilization of metals from this fraction. Exchangeable metal ions are a measure of those trace metals which are readily released into the environment.

Metals corresponding to the exchangeable fraction usually represent a small portion of the total metal content in soil, sewage sludges, and sediments and can be replaced by neutral salts [138,139]. Thus, this fraction generally accounts for less than 2% of the total metals in soil, the exceptions to this are the macro-elements (K, Ca and Mn) [141]. Salts solutions of $MgCl_2$, $CaCl_2$, $NaNO_3$, $Mg(NO_3)_2$, $BaCl_2$, KNO_3, $Ca(NO_3)_2$, NH_4OAc, $Sr(NO_3)_2$, NH_4NO_3 (at typically 1 mol L^{-1} concentration) are usually employed for leaching the metal fraction bound via electrostatic forces to the negative sites on the solid surface. Ammonium salts of strong acids, such as NH_4Cl or NH_4NO_3, however, can lower the pH and accelerate the hydrolysis of clays. Nitrate salts are advantageous over chloride and acetate salts, since no metal complexing takes place with the nitrate anion, and as a result, cation exchange is the only operating mechanism [134]. Acetate and chloride salts, apart from leaching the exchangeable fraction, enhance the extraction of transition metals as a result of their complexing action. Despite this drawback, $MgCl_2$ and NH_4OAc at 1 mol L^{-1} concentration are the most widely employed reagents for leaching the exchangeable fraction. The main advantage of solutions of ammonium salts over alkali and alkaline earth salts lies in the interfering effects that can result from the use of

relatively strong solutions of alkali and alkali metals in inductively coupled plasma atomic emission spectrometry/mass spectrometry and electrothermal atomic absorption spectrometry [133,142].

2.2.4.3 Acid soluble fraction

Acid soluble fraction contains the metals which are precipitated or coprecipitated with carbonate [143]. Carbonate can be an important adsorbent for many metals when organic matter and Fe-Mn oxides are less abundant [144]. The carbonate form is a loosely bound phase and liable to change with environmental conditions [145,146]. This phase is susceptible to changes with pH, being generally targeted by the use of a mild acid [147]. The acid soluble fraction in general contains a relatively small percentage of the total concentration of most metals and is significantly modified by drying but less than the first fraction [148].

Acidification that occurs in anoxic sediments during aeration, mainly affects the carbonate content because carbonates are dissolved when the sediment is acidified and therefore changes can be expected for the metals bound to carbonates such as Zn, Cd, Ni, Mn and Ca in the aerated sediment [149].

The reagent most widely employed in sequential extraction schemes for releasing the metal fraction associated to carbonates is the sodium acetate-acetic acid buffer at a 1 mol L^{-1} concentration and pH 5.0 [124] but the effect of the reagent is not limited to carbonate dissolution. Considerable amounts of specifically sorbed trace metals may be solubilized at pH 5 but also metal complexation must be assumed [150]. This reagent is capable of dissolving carbonates and dolomite without significant attack on organic matter, Fe and Mn oxides and aluminosilicates. Significant percentages of Fe and Mn have, however, been found in some extracts originating from sediments after treatment with sodium acetate-acetic acid buffer. This can be attributed to solubilization of $FeCO_3$ and $MnCO_3$ in acidic buffered solutions rather than to the attack of Fe and Mn oxides. Under more acidic conditions, the solubilization of Fe oxides can take place, and also the associated metals (e.g. Cu, Pb, Zn, etc). Generally, trace metals in the exchangeable and acid soluble fractions are considered readily and potentially available, respectively.

2.2.4.4 Reducible fraction

Hydrous oxides of manganese and iron are extracted together in this fraction which are in large proportion in soil and sediments [141], but are less abundant in sewage sludge. In principle, the reducible fraction could be split into three fractions: (i) easily reducible fraction (Mn oxides), (ii) moderately reducible fraction (amorphous Fe oxides), and (iii) poorly-reducible fraction (crystalline Fe oxides). So far, this classification is addressed in a few schemes [131,134] (see table 2). On the other hand, most schemes allow to differentiate between metals associated with moderately or easily reducible Fe-Mn oxides.

Hydroxylamine hydrochloride in nitric acid medium is the most widely used reagent for leaching the easily reducible fraction. One of the problems which appears is that it releases substantial amounts of trace elements bound to organic matter, consequently, the recovery of trace elements in the reducible fraction may be overestimated at the expense of the reducible fraction. A selective leaching of the fraction associated with amorphous Fe oxides can be attained with 1 mol L^{-1} hydroxylamine hydrochloride in acetic acid medium, which is capable of breaking the bonds between metals and amorphous Fe oxides without attacking either the silicates or the organic matter fraction, being included in the past in some extraction schemes to leach the moderately reducible fraction. When the pH of the extractant solution falls below 1.5, the reagent may partly release the metal content associated with the silicate fraction.

Another fairly selective reagent for dissolving amorphous Fe oxides is the oxalic acid/ammonium oxalate buffer (NH_4Ox/HOx) at pH 3. Nevertheless, leaching of metals associated with organic matter is likely to occur as a result of the complexing capacity of oxalate. In the latter case, extraction of metals associated with the organic matter by sodium hypochlorite prior to extraction of metals associated with the Fe-Mn oxides is recommended [129]. The selectivity of hydroxylamine at pH 1.5-2.0 is similar to that of ammonium oxalate in acid medium, but with less complexing capacity.

The dithionite-citrate system buffered with $NaHCO_3$ (DCB) is adequate for dissolving crystalline Fe oxides, and is implemented in some schemes. The main drawback displayed by this reagent is the presence of metal impurities, its purification being a difficult task. Additionally, this reagent easily attacks silicates. The ascorbic acid/ammonium oxalate reagent offers several advantages over the previous reagent, since it can be achieved with a high purity

degree, and does not attack silicates. This reagent has been included in some schemes such as that of Krishnamurti et al [134].

2.2.4.5 Oxidisable fraction

Trace metals might be associated through complexation or bioaccumulation processes with various forms of organic material such as living organisms, detritus or coatings on mineral particles. Organic substances exhibit a high degree of selectivity for divalent ions compared to mono-valent ions and in aquatic systems, the probable order of binding strength for metal ions onto organic matter being Hg>Cu>Pb>Zn>Ni>Co.

The organic fraction released in the oxidizable step is not considered very mobile or available since it is thought to be associated with stable high molecular weight humic substances that release small amounts of metals in a slow manner. This fraction was one of the smallest or even negligible in the surface horizons of all soils. However, this is an important fraction, especially in polluted sediments and sewage sludge which can even dominate trace metal distribution.

As metals bound to organic matter and sulfides can be easily released under oxidising conditions, an oxidation process is usually applied to leach metals associated with the abovementioned phases. The most common oxidants are hydrogen peroxide in acid medium, NaOCl at pH 9.5, and $Na_4P_2O_7$ (or $K_4P_2O_7$) at pH 9.5. In general, the hydrogen peroxide applied to a heated medium (e.g. 85°C) for several hours is the reagent preferred for dissolving organic matter as a compromise between a complete attack of organic matter and minimum alteration of silicates. Despite H_2O_2 heated to 85°C being a formidable oxidant of organic matter, oxidation is incomplete in the presence of high levels of organic matter for resistant organics and sulfide minerals. Perhaps the most important drawback displayed by this reagent is metal readsorption in the residual fraction which requires an additional extraction stage with ammonium acetate at pH 2. This combination has been adopted in most schemes for leaching metals associated with organic matter and sulfides. After H_2O_2 treatment, $Mg(NO_3)_2$ has also been proposed to release Cd ions sorbed onto the soil mineral components. Sodium hypochlorite (NaOCl) at pH 9.5 and dissolves neither Fe/Mn oxides nor carbonate, and was found superior to H_2O_2 in removing organic carbon. Unlike sodium hypochlorite, H_2O_2 can attack the Fe-Mn oxides, and consequently, this reagent must be applied after dissolution of the reducible phase. Moreover, H_2O_2 does not completely leach metals associated with sulfides. Other oxidising reagents,

such as H_2O_2/ascorbic acid or HNO_3+HCl can dissolve sulfides with enhanced selectivity, but, on the other hand, silicates are attacked to some extent.

Another reagent employed for leaching metals associated with organic matter is sodium or potassium pyrophosphate, which is selective for the easily soluble organic fraction (i.e. metals associated with humic and fulvic acids). Pyrophosphate solubilizes organic matter through complexation and dispersion and, while the reagent appears to be selective for organic matter, the extraction efficiency is poor. Non-oxidising reagents have also been attempted for extraction of metals associated with organic matter such as sodium dodecyl sulfate in $NaHCO_3$ mixed with an organic compound (e.g. AEDT, diaminemethane, N,N-dimethylformamide, dimethyl-sulfoxide, etc.).

3. Samples and target compounds investigated in this thesis

3.1 Fly ash

Fly ash is the finely divided residue that results from combustion of a solid fuel, such as coal, biomass or municipal solid waste. Fly ash is composed of oxides of iron, silicon, aluminium, magnesium, calcium, sodium and potassium. Along with oxides, fly ash contains trace elements (As, Sb, Cd, Cu, Cr, Ni, Zn, Mn, Hg, Pb, etc.). However, its elemental and mineralogical compositions and its physical properties are a function of the original composition of fuels and combustion temperature.

The assessment of fly ash in the prospect of road construction has to take into account their possible interactions with the environment. Figure 55 presents potential environmental interactions between fly ash and its environment in the context of road construction and use: (1) air transport of fine particle towards various targets, (2) seepage toward an ecosystem, (3) pollutant transfer to soil and underground water, (4) and (5) increased pollutant transfer to soil or underground water due to the effect of climatic conditions strains and the effect of traffic strains, respectively. Thus the potential environmental impact of contaminant leaching of organic and inorganic species is worth investigating.

Leaching studies of toxic metals provide relevant information of bioaccessible and the level of immobilization of metals fly ash in cement-based material. The concentration of heavy metals in the ash and their ability to leach into ground water is a serious concern when sitting and designing municipal solid waste incinerator (MSWI) ash landfills.

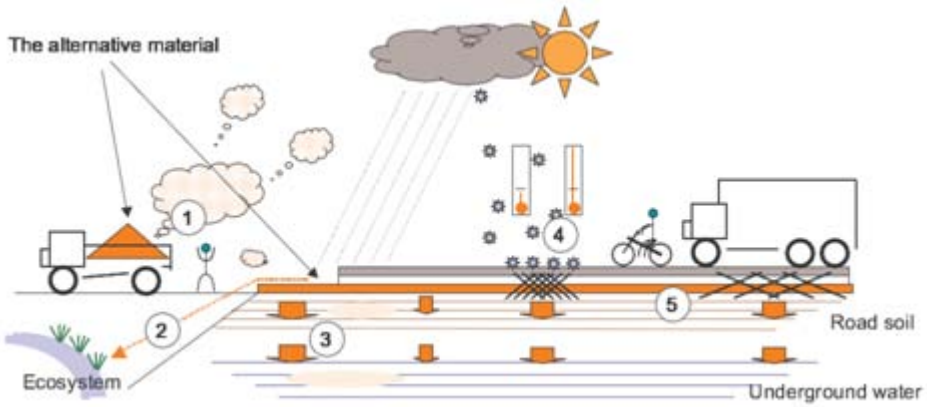


Figure 55. Potential interactions between fly ash as alternative material in the environment

3.2 Woody biomass

Biomass might be defined as organic plant or animal material that is available on a renewable basis. Biomass energy resources include food crops, grassy and woody plants, agricultural and forestry residues, municipal and industrial solid wastes, and landfill gas. These resources are renewable because although individual trees and plants are consumed, additional trees and plants can be cultivated and grown relatively quickly, and municipal and industrial solid wastes are continually produced.

Woody biomass is plant material from trees and shrubs that can include roots, bark, leaves, branches, limbs, trunks, and vines. Woody biomass might arise from many sources, including forestry operation residues, wood product residues, urban waste wood, trees grown specifically for energy, fuelwood, and forest thinnings that reduce damage from fires and pests. Woody biomass can provide a locally available, renewable source of energy that can be combined with other energy options to help meet growing energy needs in an environmentally, socially, and economically sustainable way.

3.3 Landfill leachate

Landfill leachate is generated from liquids existing in the waste as it enters a landfill or from rainwater that passes through the waste within the facility. The leachate consists of different organic and inorganic compounds that may be either dissolved or suspended. If not properly managed, the leachate is at risk for mixing with groundwater near the site, which can have dire effects. An important part of maintaining a landfill is managing the leachate through proper

treatment methods designed to prevent pollution into surrounding ground and surface waters. The most common source of landfill leachate is rainwater filtering down through the landfill and aiding bacteria in the process of decomposition. The leachate may be virtually harmless or dangerously toxic, depending upon what is in the landfill. Typically, landfill leachate has high concentrations of nitrogen, iron, organic carbon, manganese, chloride and phenols. Other chemicals, including pesticides, solvents and heavy metals, may also be present.

Frequently, mineral contamination from leachate relates to the introduction and accumulation of heavy metals in soils. Although organic contaminants generally are more degradable, the fate of their long term input in soils is not well known. Yet, such active compounds are designed to act upon soil microorganisms at low concentrations and, therefore, represent serious environmental risk.

3.4 Soil and water samples

Soils play an important role in the regulation of contaminants in ecosystems. Soil constituents can attenuate potentially toxic concentrations of contaminants and accordingly diminish the risk of surface water and groundwater contamination. For instance, silicates, oxides and organic matter can immobilize metals and adsorb organic pollutants, promoting their degradation to non-toxic forms, attenuating their movement through the soil, or preventing their uptake by plants and their introduction into the food chain. This so called “natural attenuation” and relies on natural processes to immobilize, degrade and dissipate contaminants in soil and groundwater. These processes include physical, chemical and biological mediated reactions (e.g., aerobic/anaerobic biodegradation, volatilization, oxidation, reduction, and adsorption) [151]. However, predicting the fate and behavior of organic contaminants is still difficult due to: (i) a large range of components with different properties (polarity, molecular weight, functionalities, etc.), (ii) a variety of complex dynamic physical, chemical and biological processes in environmental solid substrates and (iii) spatial heterogeneity and transient conditions in unsaturated soil zones.

3.5 Inorganic trace contaminants in environmental solids

Most metals exist naturally in the earth’s crust at trace concentrations, sufficient to provide living systems with essential nutrients but too low to cause toxicity. Since the industrial revolution, pollution by hazardous metals has substantially increased through industrial effluents and landfill leaching, mining activities, fertilizer and pesticide use in agriculture, the burning of waste and

fossil fuels, and municipal waste treatment. Since metals cannot be degraded, they are persistent and accumulate over time in the environment, including the food chain [151]. Among the transition and heavy metals cadmium, copper, lead, mercury, nickel and zinc, together with metalloid arsenic, are considered the most hazardous. Because of their threat to human health, and the extent of the problems related to both natural and anthropogenic contamination by metals and metalloids, major efforts have been undertaken to develop remediation technologies based either on physical or chemical principles, or on biological processes for the treatment of metal-contaminated soils, sediments and groundwater.

Cadmium is one of the most toxic elements with reported carcinogenic effects in humans [152]. It accumulates mainly in the kidney and liver and high concentrations have been found to lead to chronic kidney dysfunction. It induces cell injury and death by interfering with calcium (Ca) regulation in biological systems. It has been found to be toxic to fish and other aquatic organisms [152]. Cadmium has been implicated in endocrine disrupting activities, which could pose serious health problems. Apart from health implications, Cd together with other elements, e.g., Zn form a toxic that often acts synergistically. Sources of Cd include wastes from Cd-based batteries, incinerators and runoff from agricultural soils where phosphate fertilizers are used since Cd is a common impurity in phosphate fertilizers [153].

The United States Environmental Protection Agency has classified Pb as being potentially hazardous and toxic to most forms of life. It has been found to be responsible for quite a number of ailments in humans such as chronic neurological disorders especially in foetuses and children. Automobile exhaust fumes have been reported in the past to account for about 50% of the total inorganic Pb absorbed by human beings [154]. Other inputs of Pb into the environment are from used dry-cell batteries, from sewage effluent, runoff of wastes and atmospheric deposition.

Although Zn has been found to have low toxicity to human beings, prolonged consumption of large doses can result in some health complications such as fatigue, dizziness, and neutropenia and also Zn could be toxic to some aquatic organisms such as fish. Certain metals such as Cu and Co are classified as essential to life due to their involvement in certain physiological processes. Elevated levels of these, however, have been found to be toxic. Toxicological effects of large amounts of Co include for example vasodilation, flushing and cardiomyopathy in humans and animals [155].

Nickel also is a naturally occurring element found in a number of mineral ores including nickel sulphides, oxides and silicates. It is present in the enzyme urease and as such is considered to be essential to plants and some domestic animals. The essentiality of Ni to human beings has not been yet demonstrated [155]. Its properties such as strength, corrosion resistance, high ductility, good thermal and electric conductivity and catalytic properties enhance its commercial importance and applications. Toxic effects in human beings are related to dermal, lung and nasal sinus cancers.

3.6 Organic trace level contaminants

Persistent Organic Pollutants (POPs) are a set of chemicals that are toxic, persist in the environment for long periods of time, and biomagnified as they move up through the food chain. POPs have been linked to adverse effects on human health and animals, such as cancer, damage to the nervous system, reproductive disorders, and disruption of the immune system. Because they circulate globally via the atmosphere, oceans, and other pathway. POPs released in one part of the world can travel to regions far from their source of origin. Organic chemicals enter the unsaturated soil environment through various anthropogenic activities including accidental spills, leaking underground storage tanks, improper waste disposal and landfill leachate. When organic chemicals are released to the soil, they enter a three-dimensional heterogeneous environment composed of soil minerals, soil organic matter, soil water, soil vapors and biota. All these compartments vary in size, form and composition. Thus, small scales and spatial heterogeneity of soil makes the estimations of physical and physico-chemical conditions extremely challenging [156]. The range of components is large and they interact with each other in complex ways. However, the fate and behavior of a specific organic chemical depends primarily on three basic processes: (i) retention, (ii) degradation, and (iii) transportation (Fig. 56). Both, the direction and rate of these processes are strictly dependent on the chemical nature of the contaminant and the physical, chemical, biological, and hydraulic properties of the soil. These processes can be grouped into those that affect persistence, including chemical and microbial degradation, and those that affect mobility and bioaccessibility, involving sorption, plant uptake, volatilization, wind erosion, run-off and leaching. Accordingly, the fate of a contaminant can be predicted to a some extent by knowing selected properties of both the organic contaminants and the adsorbents.

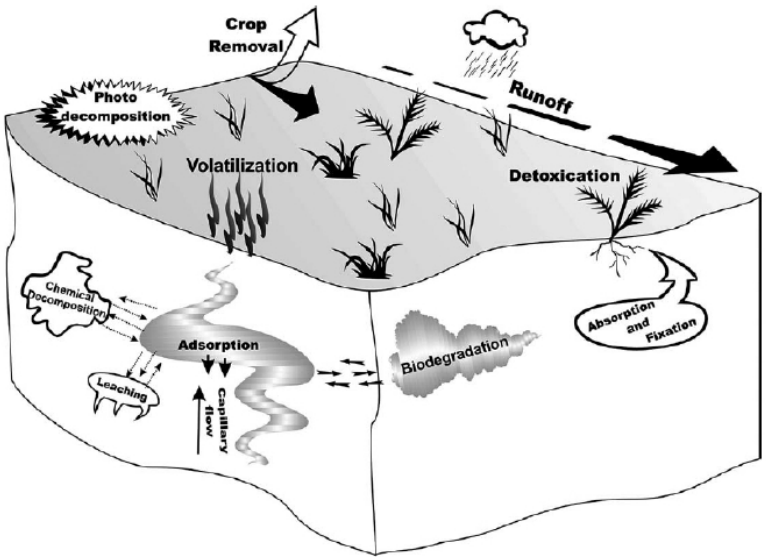


Figure 56. Fate of organic contaminant residues in soil environments [157].

3.6.1 Triazine and its metabolites

Triazine class compounds, selective systemic nitrogenated herbicides, have been widely used in recent years for weed control in several crops. Due to their persistence, triazine residues may remain in these crops, which is of public concern due to the negative effects they might have on human health. In fact, some European countries have included simazine and atrazine, the most widely used triazines, on the list of pesticide residues to be controlled and have established the corresponding maximum residue limits (MRLs) allowed in food. The MRLs for these triazines vary within the 0.02 to 0.1 mg kg^{-1} range depending on the vegetable, and it is therefore necessary to use analytical methods capable of detecting the presence of these herbicides at this low concentration level. Atrazine and its metabolites have been found in surface water and groundwater as a result of the use of atrazine as a pre-emergent or early post-emergent herbicide. The source of the residue should be considered when interpreting water monitoring data. Assessments should be based on whether diffuse sources (e.g. runoff from an agricultural field) or point sources (e.g. an accidental spill or inappropriate disposal) have contributed to a detection in water. The presence of atrazine and its metabolites in surface water is most likely to be intermittent, particularly in flowing water, but groundwater contamination will usually be relatively persistent.

A great majority of triazine herbicides are derived from *s*-triazine, a six-membered heterocycle with symmetrically located nitrogen atoms, that is substituted in positions 2, 4 and 6 (see Fig. 57).

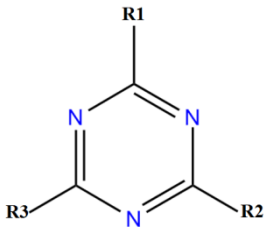


Figure 57. Chemical structure of *s*-triazine with substituent positions [158]

Unsubstituted *s*-triazine is somewhat similar to benzene, however, its stability is lower because the perfectly delocalized π-bond system of benzene is disturbed by the introduction of nitrogen atoms to positions 1, 3 and 5, with a subsequent increase in the electron density in these positions and a corresponding decrease in the electron density in positions 2, 4 and 6; nucleophilic substitution in the latter positions is thus facilitated. Nevertheless, the stereochemical stability of *s*-triazines is still large and the persistence of triazine pesticides and their degradation products in soils, waters and plant and animal materials is considerable, from several months for the parent compounds to many years for their degradation products, which might be even more toxic. The physico-chemical properties of *s*-triazine derivatives are primarily determined by the substituent in position 2, this is most often -Cl (the commercial names ending with -azine), $-SCH_3$ (-tryn) and $-OCH_3$ (-ton). The properties of chloroderivatives considerably differ from those of the other two types, whereas the thiomethyl- and methoxy-derivatives behave very similarly. Positions 4 and 6 are usually occupied by substituted amino groups and exert substantially smaller effects on the derivative properties. A list of common *s*-triazine derivatives and some of their properties are given in Table 3 and the toxicity levels are listed in Table 4. As can be seen, *s*-triazines are weakly basic, poorly water soluble compounds of a low polarity, stable both in the solid phase and in solution. The basicity increases with the order of substituents in position 2 of $Cl<SCH_3<OCH_3$; the substituents in positions 4 and 6 affect the basicity to a lesser degree, but the basicity increases with an increasing number of hydrogen atoms in the substituted amino group and with increasing length and branching of the alkyl chain. The solubility in water increases with increasing acidity, due to protonation. Hydrolysis occurs in strongly acidic and basic solutions, especially at elevated temperatures; hydroxy derivatives are formed but the *s*-triazine nucleus is preserved.

Table 3 selected characteristics of some *s*-triazine herbicides and their derivatives

compound	substituents			pK_a	λ_1	ε_1	λ_2	ε_2	Log $P_{ow/w}$
	R_1	R_2	R_3		nm	L mol^{-1} cm^{-1}	nm	L mol^{-1} cm^{-1}	
Simazine	Cl	NHC_2H_5	NHC_2H_5	1.65	222	36000	263	3100	2.3
Atrazine	Cl	NHC_2H_5	$NHCH(CH_3)_2$	1.68	222	41000	263	3900	2.7
Propazine	Cl	$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	1.85	221	32000	268	3100	2.91
Terbutylazine	Cl	NHC_2H_5	$NHC(CH_3)_3$	1.94	223	19500	263	1800	3.06
Trietazine	Cl	NHC_2H_5	$N(C_2H_5)_2$	1.88	227	44300	267	4300	3.07
Ipazine	Cl	$N(C_2H_5)_2$	$NHCH(CH_3)_2$	1.85	228	43100	266	4300	
Deethylatrazine	Cl	NH_2	$NHCH(CH_3)_2$	1.3					1.6
Deisopropylatrazine	Cl	NHC_2H_5	NH_2	1.3					1.2
Deethyldeisopropylatrazine	Cl	NH_2	NH_2	1.5					0
Hydroxysimazine	OH	NHC_2H_5	NHC_2H_5		218				
Hydroxyatrazine	OH	NHC_2H_5	$NHCH(CH_3)_2$	5.15	218				1.4
Hydroxypropazine	OH	$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	5.2	217				
Hydroxydeethylatrazine	OH	NH_2	$NHCH(CH_3)_2$	4.75	212				0.2
Hydroxydeisopropylatrazine	OH	NHC_2H_5	NH_2	4.65	213				-0.1
Simeton	OCH_3	NHC_2H_5	NHC_2H_5	4.15	222	39300			
Atraton	OCH_3	NHC_2H_5	$NHCH(CH_3)_2$	4.2	217				2.99
Desmetryn	SCH_3	$NHCH_3$	$NHCH(CH_3)_2$	3.93	221	33700			
Simetryn	SCH_3	NHC_2H_5	NHC_2H_5	4.0	222	44400			2.8
Ametryn	SCH_3	NHC_2H_5	$NHCH(CH_3)_2$	4.0	222	40000			3.07
Prometryn	SCH_3	$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	4.05	223	42000			3.34
Terbutryn	SCH_3	NHC_2H_5	$NHC(CH_3)_3$	4.4	223	21200			3.74

λ: Absorption maximum coefficient, ε: corresponding absorption coefficient, $P_{ow/w}$: Partition coefficient between octanol and water and water

Table 4 Toxicity of selected triazine

Triazine	LD_{50} for rate (mg/kg body weight)
Deethylatrazine	710
Deisopropylatrazine	1240
Atrazine	1869-3080
Simazine	>5000
Propazine	>7700

LD_{50}: median lethal dose for the low acute toxicity in rats exposed orally

3.6.2 Polychlorinated biphenyls (PCBs)

PCBs are aromatic, synthetic chemicals which consist of the biphenyl structure with two linked benzene rings in which some or all of the hydrogen atoms have been substituted by chlorine atoms. The basic molecular structure, including the conventional numbering of the substituent positions, is shown in Fig. 58.

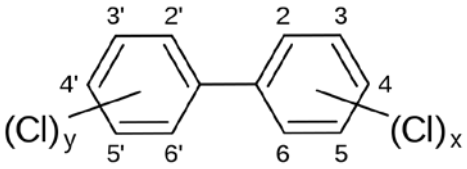


Figure 58. Chemical structure of PCBs

The chemical formula of PCBs is $C_{12}H_{10-n}Cl_n$, where n ranges from 1 to 10. PCBs enter the environment as mixtures containing a variety of individual chlorinated biphenyl components, known as congeners, as well as impurities. Theoretically, 209 different congeners are possible, but only about 130 of these have been identified in commercial products proposed a numbering system for the PCB congeners which has been adopted by the IUPAC (see Table 5). All congeners of PCBs are lipophilic and their lipophilicity increases with increasing degree of chlorination. Congeners with a lower degree of chlorination are more volatile than those with a higher degree. Pure individual PCB congeners are colourless and often crystalline. Commercial PCB mixtures are clear to light yellow oils and they do not crystallize, even at low temperatures.

Before 1977, PCBs entered the air, water, and soil during their manufacture and worldwide use. Wastes that contained PCBs were generated at that time, and these wastes were often placed in landfills. PCBs also entered the environment from accidental spills and leaks during the transport

of the chemicals, or from leaks or fires in transformers, capacitors, or other products containing PCBs. Today, PCBs can still be released into the environment from poorly maintained hazardous waste sites that contain PCBs, illegal or improper dumping of PCB wastes, such as old transformer fluids; leaks or releases from electrical transformers containing PCBs; and disposal of PCB-containing consumer products into municipal or other landfills not designed to handle hazardous waste. PCBs may be released into the environment by the burning of some wastes in municipal and industrial incinerators as well.

Once in the environment, PCBs do not readily break down and therefore may remain for very long periods of time, generally, the more chloride atoms that the PCBs contain, the more slowly they break down. They can easily cycle between air, water, and soil. For example, PCBs can enter the air by evaporation from both soil and water. In air, PCBs can be carried long distances and have been found in snow and sea water in areas far away from where they were released into the environment. In general, the lighter the type of PCBs, the further they may be transported from the source of contamination. PCBs are present as solid particles or as a vapor in the atmosphere. They will eventually return to land and water by settling as dust or in rain and snow. In water, PCBs may be transported by currents, attach to bottom sediment or particles in the water, or evaporate into air. Heavy congeners of PCBs are more likely to settle into sediments while lighter PCBs are more likely to evaporate to air and accumulate in the leaves and above ground parts of plants and food crops. Sediments that contain PCBs can also release the PCBs into the surrounding water. PCBs stick strongly to soil and will not usually be carried deep into the soil with rainwater.

Concentrations of PCBs in subsurface soil at a highly contaminated site have been as high as 750 mg L^{-1}. People who live near hazardous waste sites may be exposed to PCBs by consuming PCB contaminated sportfish and game animals, by breathing PCBs in air, or by drinking PCB-contaminated well water. Adults and children may come into contact with PCBs when swimming in contaminated water and by accidentally swallowing water during swimming. However, both of these exposures are far less serious than exposures from ingesting PCB-contaminated food (particularly sportfish and wildlife) or from breathing PCB contaminated air.

Table 5 IUPAC numbers and chloride atom positions of all PCB congeners

No.	Structure	No.	Structure	No.	Structure	No.	Structure
1	2	56	2,3,3′,4′	111	2,3,3′,5,5′	166	2,3,4,4′,5,6
2	3	57	2,3,3′,5	112	2,3,3′,5,6	167**	2,3′,4,4′,5,5′
3	4	58	2,3,3′,5	113	2,3,3′,5′,6	168	2,3′,4,4′,5′,6
4	2,2′	59	2,3,3,6	114**	2,3,4,4′,5	169*	3,3′,4,4′,5,5′
5	2,3	60	2,3,4,4′	115	2,3,4,4′,6	170***	2,2′,3,3′,4,4′,5
6	2,3′	61	2,3,4,5	116	2,3,4,5,6	171	2,2′,3,3′,4,4′,6
7	2,4	62	2,3,4,6	117	2,3,4′,5,6	172	2,2′,3,3′,4,5,5′
8	2,4′	63	2,3,4′,5	118**	2,3′,4,4′,5	173	2,2′,3,3′,4,5,6
9	2,5	64	2,3,4′,6	119	2,3′,4,4′,6	174	2,2′,3,3′,4,5,6′
10	2,6	65	2,3,5,6	120	2,3′,4,5,5′	175	2,2′,3,3′,4,5′,6
11	3,3′	66	2,3′,4,4′	121	2,3′,4,5′,6	176	2,2′,3,3′,4,6,6′
12	3,4	67	2,3′,4,5	122	2′,3,3′,4,5	177	2,2′,3,3′,4′,5,6
13	3,4′	68	2,3′,4,5′	123**	2′,3,4,4′,5	178	2,2′,3,3′,5,5′,6
14	3,5	69	2,3′,4,6	124	2′,3,4,5,5′	179	2,2′,3,3′,5,6,6′
15	4,4′	70	2,3′,4′,5	125	2′,3,4,5,6′	180***	2,2′,3,4,4′,5,5′
16	2,2′,3	71	2,3′,4′,6	126*	3,3′,4,4′,5	181	2,2′,3,4,4′,5,6
17	2,2′,4	72	2,3′,5,5′	127	3,3′,4,5,5′	182	2,2′,3,4,4′,5,6′
18	2,2′,5	73	2,3′,5′,6	128	2,2′,3,3′,4,4′	183	2,2′,3,4,4′,5′,6
19	2,2′,6	74	2,4,4′,5	129	2,2′,3,3′,4,5	184	2,2′,3,4,4′,6,6′
20	2,3,3′	75	2,4,4′,6	130	2,2′,3,3′,4,5′	185	2,2′,3,4,5,5′,6
21	2,3,4	76	2′,3,4,5	131	2,2′,3,3′,4,6	186	2,2′,3,4,5,6,6′
22	2,3,4′	77*	3,3′,4,4′	132	2,2′,3,3′,4,6′	187	2,2′,3,4′,5,5′,6
23	2,3,5	78	3,3′,4,5	133	2,2′,3,3′,5,5′	188	2,2′,3,4′,5,6,6′
24	2,3,6	79	3,3′,4,5′	134	2,2′,3,3′,5,6	189**	2,3,3′,4,4′,5,5′
25	2,3′,4	80	3,3′,5,5′	135	2,2′,3,3′,5,6′	190	2,3,3′,4,4′,5,6
26	2,3′,5	81	3,4,4′,5	136	2,2′,3,3′,6,6′	191	2,3,3′,4,4′,5′,6
27	2,3′,6	82	2,2′,3,3′,4	137	2,2′,3,4,4′,5	192	2,3,3′,4,5,5′,6
28	2,4,4′	83	2,2′,3,3′,5	138	2,2′,3,4,4′,5′	193	2,3,3′,4′,5,5′,6
29	2,4,5	84	2,2′,3,3′,6	139	2,2′,3,4,4′,6	194	2,2′,3,3′,4,4′,5,5′
30	2,4,6	85	2,2′,3,4,4′	140	2,2′,3,4,4′,6′	195	2,2′,3,3′,4,4′,5,6
31	2,4′,5	86	2,2′,3,4,5	141	2,2′,3,4,5,5′	196	2,2′,3,3′,4,4′,5,6′
32	2,4′,6	87	2,2′,3,4,5′	142	2,2′,3,4,5,6	197	2,2′,3,3′,4,4′,6,6′
33	2′,3,4	88	2,2′,3,4,6	143	2,2′,3,4,5,6′	198	2,2′,3,3′,4,5,5′,6
34	2′,3,5	89	2,2′,3,4,6′	144	2,2′,3,4,5′,6	199	2,2′,3,3′,4,5,6,6′
35	3,3′,4	90	2,2′,3,4′,5	145	2,2′,3,4,6,6′	200	2,2′,3,3′,4,5′,6,6′
36	3,3′,5	91	2,2′,3,4′,6	146	2,2′,3,4′,5,5′	201	2,2′,3,3′,4′,5,5′,6
37	3,4,4′	92	2,2′,3,5,5′	147	2,2′,3,4′,5,6	202	2,2′,3,3′,5,5′,6,6′
38	3,4,5	93	2,2′,3,5,6	148	2,2′,3,4′,5,6′	203	2,2′,3,4,4′,5,5′,6
39	3,4′,5	94	2,2′,3,5,6′	149	2,2′,3,4′,5′,6	204	2,2′,3,4,4′,5,6,6′
40	2,2′,3,3′	95	2,2′,3,5′,6	150	2,2′,3,4′,6,6′	205	2,3,3′,4,4′,5,5′,6
41	2,2′,3,4	96	2,2′,3,6,6′	151	2,2′,3,5,5′,6	206	2,2′,3,3′,4,4′,5,5′,6
42	2,2′,3,4′	97	2,2′,3′,4,5	152	2,2′,3,5,6,6′	207	2,2′,3,3′,4,4′,5,6,6′
43	2,2′,3,5	98	2,2′,3′,4,6	153	2,2′,4,4′,5,5′	208	2,2′,3,3′,4,5,5′,6,6′
44	2,2′,3,5′	99	2,2′,4,4′,5	154	2,2′,4,4′,5,6′	209	2,2′,3,3′,4,4′,5,5′,6,6′
45	2,2′,3,6	100	2,2′,4,4′,6	155	2,2′,4,4′,6,6′		
46	2,2′,3,6′	101	2,2′,4,5,5′	156**	2,3,3′,4,4′,5		
47	2,2′,4,4′	102	2,2′,4,5,6′	157**	2,3,3′,4,4′,5′		
48	2,2′,4,5	103	2,2′,4,5′,6	158	2,3,3′,4,4′,6		
49	2,2′,4,5′	104	2,2′,4,6,6′	159	2,3,3′,4,5,5′		
50	2,2′,4,6	105*	2,3,3′,4,4′	160	2,3,3′,4,5,6		
51	2,2′,4,6′	106	2,3,3′,4,5	161	2,3,3′,4,5′,6		
52	2,2′,5,5′	107	2,3,3′,4′,5	162	2,3,3′,4′,5,5′		
53	2,2′,5,6	108	2,3,3′,4,5′	163	2,3,3′,4′,5,6		
54	2,2′,6,6′	109	2,3,3′,4,6	164	2,3,3′,4′,5′,6		
55	2,3,3′,4	110	2,3,3′,4,6	165	2,3,3′,5,5′,6		

*non-ortho congener, ** mono-ortho congener, ***di-ortho congener.

These congeners are also chlorinated in both *para*- and at least two *meta*- positions.

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CHAPTER 2

AIMS OF THESIS

Chapter 2

AIMS OF THESIS

The goal of this study is to develop and apply novel automated sample preparation methods for the determination of trace level concentrations of organic and inorganic pollutants in environmental sample exploiting flow-based techniques. The first part of this thesis is dedicated to design dynamic leaching/fractionation methods as a front end to inorganic elemental detection systems for assessing metals mobility and bioaccessibility in environmental solids and industrial solid wastes. Experimental results and discussion are complied in Chapters 3-6.

The second part involves developing on-line sample preparation methods for extraction, preconcentration and determination of organic pollutants at concentration levels lower than those endorsed in current EU Directives as presented in Chapters 7-9.

Specific aims of this dissertation are given below and itemized in the various chapters.

Chapter 3. Multiple stirred-flow chamber assembly for simultaneous automatic fractionation of trace elements in fly ash samples using a multisyringe-based flow system

- Set-up robust and fully automated stirred-flow cell extractors.
- Investigate the leaching of trace metals (namely, Cu, Cd, Ni, Pb and Zn) from coal fly ash under worst-case scenarios exploiting the Toxicity Characteristic Leaching Procedure (TCLP) for solid wastes.
- Design a parallel arrangement for simultaneous multiple dynamic extractions for routine evaluation of potential reuse of fly ash.

Chapter 4. Critical evaluation of novel dynamic flow-through methods for automatic sequential BCR extraction of trace metals in fly ash

- Compare 2 dynamic leaching methods, *namely*, sequential injection microcolumn extraction (SI-MC) and sequential injection stirred-flow cell extraction (SI-SFC) for characterization of fly ash.

- Demonstrate that the solid-to-liquid ratio is not a critical parameter for dynamic leaching in comparison with batchwise extraction procedures.

- Chemical fractionate and determine Cu, Cd, Ni, Pb and Zn in fly ash using the European standard BCR method.

- Set the basis for harmonization of dynamic leaching tests.

Chapter 5. Automated dynamic chemical fractionation method with detection by plasma spectrometry for advanced characterization of solid biofuels

- Set up characterize and optimize an automatic system involving on-line coupling of dynamic chemical fractionation to ICP-OES for advance characterization of woody biomass fuels.

- Determine mobile forms of ash-forming elements (Na, Ca, Mg, K) under worst case conditions in biofuels to estimate the level of alkali and alkaline elements participating in slagging and fouling of boilers and fluidized bed reactors.

Chapter 6. Elucidation of associations of ash-forming matter in woody biomass residues

- Elucidate of metal-biomass/ash associations and investigation of the actual selectivity of extractants with combination of dynamic extraction approach and characteristic techniques including scanning electron microscopy with energy dispersive X-ray fluorescence spectrometry (SEM-EDX) and X-ray diffraction (RXD) assays for biofuels application.

Chapter 7. Online hyphenation of multimodal microsolid phase extraction involving renewable molecularly imprinted and reversed-phase sorbents to liquid chromatography for automatic multiresidue assays

- Set up characterize and optimize an automatic system involving on-line hyphenation of multimodal μSPE consisting of renewable MIPs and reversed-phase sorbents (Oasis HLB) to LC.
- Perform selective preconcentration and determination of chlorotriazines and their metabolites in environmental samples at concentration levels lower than those endorsed in the current EU Directives Water Framework.

Chapter 8. In-line stirred-flow sorptive microreactor for automatic preconcentration of priority organic pollutants using carbon nanostructured materials prior to liquid chromatographic separation

- Design a novel flow-through arrangement for expedient handling of carbon nanoparticles in flow systems.
- Hyphenate on-line μSPE using cup-stacked nanotubes as sorbent materials to HPLC for determination of trace level concentrations of priority pesticides in soil extracts and ground waters samples.

Chapter 9. On-line coupling of bead injection-lab on valve analysis to gas chromatography (BI-LOV-GC): Application to the determination of trace levels of polychlorinated byphenyls (PCBs) in solid waste leachate samples

- Interface bead injection in a lab-on-valve format to GC for automatic μSPE and eluate analysis using large volume injection.
- Investigate analytical parameters for reliable and sensitive assays of PCBs in troublesome solid waste leachates.

CHAPTER 3

Multiple stirred-flow chamber assembly for simultaneous automatic fractionation of trace elements in fly ash samples using a multisyringe-based flow system

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Multiple Stirred-Flow Chamber Assembly for Simultaneous Automatic Fractionation of Trace Elements in Fly Ash Samples Using a Multisyringe-Based Flow System

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There is a current trend in automation of leaching tests for trace elements in solid matrixes by use of flow injection based column approaches. However, as a result of the downscaled dimensions of the analytical manifold and execution of a single extraction at a time, miniaturized flow-through column approaches have merely found applications for periodic investigations of trace element mobility in highly homogeneous environmental solids. A novel flow-based configuration capitalized on stirred-flow cell extraction is proposed in this work for simultaneous fractionation of trace elements in three solid wastes with no limitation of sample amount up to 1.0 g. A two-step sequential extraction scheme involving water and acetic acid (or acetic acid/acetate buffer) is utilized for accurate assessment of readily mobilizable fractions of trace elements in fly ash samples. The fully automated extraction system features high tolerance to flow rates ($\leq$6 mL min^{-1}) and, as opposed to operationally defined batchwise methods, the solid to liquid ratio is not a critical parameter for determination of overall readily leachable trace elements provided that exhaustive extraction is ensured. Analytical performance of the dynamic extractor is evaluated for fractionation analysis of a real coal fly ash and BCR-176R fly ash certified reference material. No significant differences were found at the 0.05 significance level between summation of leached concentrations in each fraction plus residue and concentration values of BCR-176R, thus revealing the accuracy of the automated method. Overall extractable pools of trace metals in three samples are separated in less than 115 min, even for highly contaminated ashes, versus 18−24 h per fraction in equilibrium leaching tests. The multiple stirred-flow cell assembly is thus suitable for routine risk assessment studies of industrial solid byproduct.

Fly ash is the finely divided residue that results from combustion of a solid fuel, such as coal[1] or municipal solid waste.[2] Over the past decade there has been a vast interest in reutilization of fly ash in civil engineering works as an inexpensive replacement of Portland cement because of increased life of roads and structures by improving concrete durability.[1,3] The elemental and mineralogical compositions and physical properties of fly ashes are a function of original coal and combustion temperature, yet the material is always regarded as a hazardous waste owing to the potentially toxic trace metal content.

Batchwise leaching tests are the choice tools for detection of potential hazardous effects resulting from disposal or reuse of solid wastes because relevant knowledge as to mobility, availability, and/or eventual impact of anthropogenic metal ions on ecological systems and biological organisms can be drawn.[4,5] A survey of literature revealed the existence of a wealth of extraction protocols currently applied for fractionation of trace elements in fly ash[6−10] based on different sequence schemes and carried out under various operationally defined conditions. Common to all fractionation schemes of solid materials is the subjection of a certain amount of collected ash to the action of increasingly aggressive leaching agents aimed at releasing particular metal fractions into the liquid phase under steady-state conditions.

Even though batchwise extraction methods are well accepted for environmental risk exposure of trace elements in environmental substrates, the ecotoxicological relevance of the information provided is, in fact, questionable since natural occurring processes are always dynamic, while manual protocols, intended to simulate environmental scenarios, inherently are based upon the establishment of a single equilibrium between solid and liquid phases.[11]

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Two additional major problems have been also recognized,[5,12,13] that is, the lack of selectivity of leaching agents for releasing metals associated with a discrete geological phase which is to be influenced by the extractant exposure time and redistribution of target species among phases during extraction, that is, trace elements released by one extractant could associate with other undissolved solid components or freshly exposed surfaces within the time-scale of the extraction step.

In order to alleviate the above drawbacks and gain full knowledge on the kinetics of the metal leaching processes, recent trends have been focused on the development of flow-through dynamic (nonequilibrium) partitioning methods to better simulate the percolation of rainwater through environmental solid profiles.[11] The fundamental principles of these novel extraction strategies, mostly based on the various generations of flow injection analysis, are critically discussed in a recent review article.[14] Most of these approaches integrate a microcolumn packed with a given amount of solid sample at the low milligram level (typically 5−50 mg)[15–19] within the flow network through which defined volumes of extractants are pumped either in a continuous-flow or flow-programming fashion.[15−22] The most severe limitations are the small inner capacity of microcartridges and backpressure increase when using sample weights above 100 mg, whereby the technique is merely suitable for fractionation of highly homogeneous samples, otherwise sample representativeness might not be assured. One attempt to expand the scope of dynamic extraction was to design semiautomatic stirred-flow cell extractions where large amounts of sample (>250 mg) could be handled.[23] However, this concept was proven unsuitable for fractionation schemes because of the lack of steady leachant flow rate throughout the extraction protocol.[23] Regular recalibration of the peristaltic pump used for delivery of extractants or frequent renewal of flexible Tygon pump tubing was actually imperative.[23,24]

Despite the fact that flow-through methods are reported to greatly accelerate leaching tests,[15–22] as opposed to batchwise end-over-end method, samples are processed one at a time. This is indeed the major shortcoming of flow-through extractors coupled in-line to atomic absorption or atomic emission spectrometers.[15–17,19–21] To make dynamic extractions/fractionations practical for routine analysis, there is a need for development of novel instrumentation capable of performing sequential extractions in a parallel fashion.

To this end, a fully automated flow-through extraction assembly capitalized on coupling multisyringe pumping with stirred-flow chambers is proposed in this work for reliable and simultaneous execution of multiple extractions. To the best of our knowledge, no analytical setup for parallel dynamic fractionation of environmental samples has been reported so far. By replacing peristaltic pumps with rugged liquid drivers (namely, syringe pumps), the hyphenated extractor is capable of enduring the flow backpressure caused by fine particle clogging of membrane filters of sample containers. The devised system was exploited for expeditious investigation of the leaching behavior of trace elements in fly ash using a two-step sequential extraction protocol. This is the first application of fully automated flow-through extraction for evaluation of potentially mobilizable pools of trace elements in fly ash. Distilled water as endorsed in the European standard EN 12457[25] and the German DIN 38414-S4[26] tests was used as the first leaching solution for ascertainment of the most ecotoxicological relevant fraction, that is, the water soluble elements. Acetic acid or acetic acid/acetate buffer is utilized as the second extractant following the guidelines of the toxicity characteristic leaching procedure (TCLP)[27] for ascertainment of increased release of metals by natural or anthropogenic acidification processes, such as acid spill or acid rainfall. The analytical performance of the parallel flow-through extractor is explored for dynamic fractionation of Cu, Cd, Ni, Pb, and Zn in coal fly ash. Method validation is accomplished through the use of mass balance and analysis of a certified municipal solid waste incineration (MSWI) fly ash material (BCR-176R).

EXPERIMENTAL SECTION

Reagents and Solutions. All reagents were of analytical grade and Milli-Q water (Milllipore Synthesis A10, France) was used throughout. The acetic acid extractant (pH 2.88) was prepared by dilution of 5.7 mL of glacial acetic acid (Scharlau Chemie, Spain) with water to a volume of 1 L. Acetic acid/sodium acetate buffer solution (pH 4.93) was prepared by adding 5.7 mL of glacial acetic acid and 64.3 mL of 1.0 mol L^{-1} NaOH to 500 mL of water and diluting to a volume of 1 L. Ortho-phosphoric acid (Scharlau Chemie), nitric acid (Merck, Germany), and fluoroboric acid (Probus, Spain) were used for microwave digestion. ICP-multi-element standard solution XI (AccuTrace Reference Standard, AccuStandard, CT) was employed for external calibration. A matrix matched procedure was used for analyses of water, acetic acid, and acetic acid/acetate buffer leachates.

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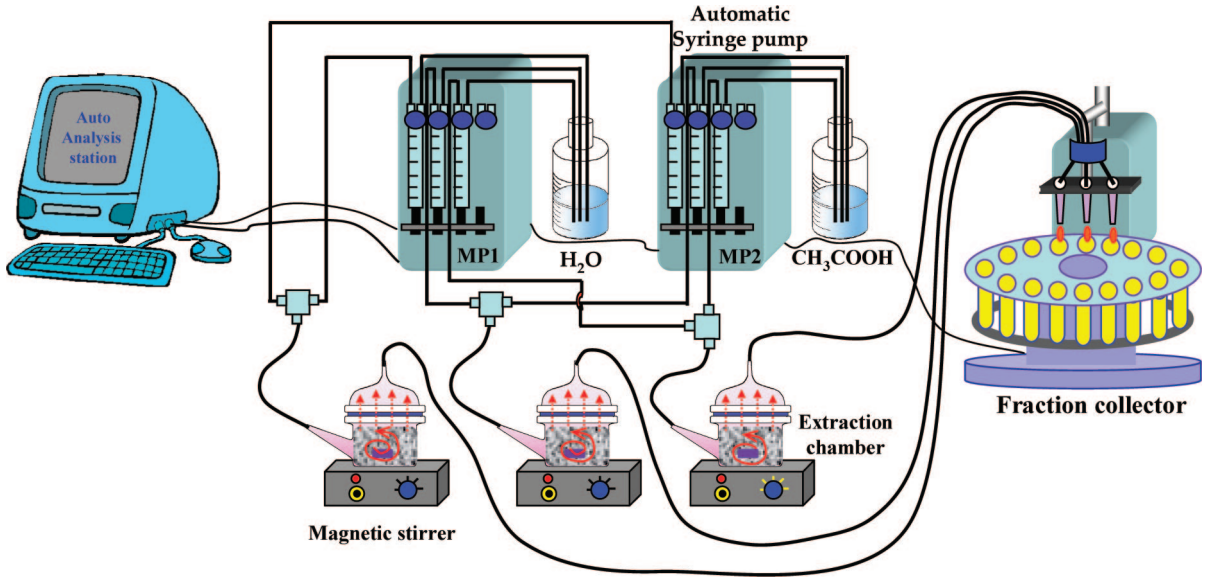

Figure 1. Multisyringe stirred-flow cell setup for three simultaneous flow-through leaching tests.

Polyethylene containers were cleansed in a 10% (v/v) HNO_3 bath overnight and then washed successively with deionized water prior to use.

Multisyringe Stirred Flow Cell Extractor. The miniaturized device for dynamic fractionation of multiple solid samples is composed of two multisyringe pump modules (BU 4S, Crison Instruments, Allela, Spain) hyphenated to three stirred-flow extraction chambers and one or two 45-position rack autosamplers (Micro Sampler, Crison Instruments). The piston pumps are furnished with 10-mL glass syringes of 10 mL each which are connected in block to a single stepper motor, whereby three extractant solutions might be simultaneously delivered to the extraction chambers at will. Each syringe is equipped with a two-way solenoid commutation valve, which allows connection with the sample container or reagent reservoir regardless of piston displacement.

The extraction chambers were designed to contain a weighed sample (<2.0 g) and to allow extractants to flow sequentially to successively leach metals from the targeted phases of the stirred fly ash-extract mixture. The extraction chamber and its cover were constructed from borosilicate glass to have a capacity of ~10 mL, as described elsewhere.[23] A rubber gasket was placed on top of the chamber followed by a nylon filter (GE Osmonics Labstore, MN) of 0.45 μm pore size and 47 mm diameter to allow dissolved matter to flow through but retain ash particles. The setup is completed with a second rubber gasket and the cover on top of the extraction chamber. The inlet of the chamber is connected to the syringe pumps while the outlet to the autosampler devices using small pieces of Tygon tube, PTFE tubing of 1.5 mm i.d., and PVC T-connectors. A schematic illustration of the multisyringe stirred-flow cell extractor is shown in Figure 1. The autosampler arm has been adapted for simultaneous accommodation of three lines aimed at parallel extractant collection. The extracts were automatically collected in 12-mL plastic containers placed at given autosampler locations. Whenever required, a second autosampler can be attached in parallel using three ancillary solenoid valves (not shown in Figure 1) as interfaces between the outlets of the chambers and leachate collectors.

Automated control of the backward/forward motion of the syringe pumps, extractant delivery flow rate, and position of autosampler containers were performed via the dedicated software package AutoAnalysis 5.0 (Sciware, Spain). The software based on dynamic link libraries (DLLs) is composed of a single and versatile protocol wherein the piston pump and autosampler DLLs are for this given application attached.

Sample Collection and Characterization. Coal fly ash was collected from the hoppers of the electrostatic precipitator of the power generating plant of the Electricity Generating Authority of Thailand in Mae Moh at Lumpang province, Thailand. The coal fly ash sample was dried at 105 °C until constant weight, stored in clean polyethylene bags, and kept in a dry place.

Coal fly ash was classified for particle size distribution by a sieving method using different sieves (Endecotts, U.K.), namely, 20, 45, 150, and 300 μm, respectively. Particle size range of Mae Moh fly ash is 2.7%, 24%, 61.9%, 10.6%, and 0.8% (w/w) within the range of less than 20, 20−45, 45−150, 150−300, and higher than 300 μm, respectively. The color of the coal fly ash particle differs from one size to another. Brownish-gray particles are mainly found for particles of <20 μm whereas grayish-black particles are observed for larger particles.

Determination of major elements in Mae Moh coal fly ash was performed by an X-ray fluorescence spectrometer (S4 Explorer, Bruker, Germany). Coal fly ash is mainly composed of 35.2% (w/w) quartz (SiO_2), 21.7% (w/w) alumina (Al_2O_3), 15.4% (w/w) hematite (Fe_2O_3), and 15.0% (w/w) calcite ($CaCO_3$, present as CaO). Significant percentages of K_2O, MgO, Na_2O, and P_2O_5 are also found in this sample. Morphology of Mae Moh coal fly ash was investigated by use of optical microscopy (ZEISS Microscope with KS300 software), and particles were found to be spherically shaped.

According to XRF results and chemical requirements of ASTM C618,[3] it can be concluded that Mae Moh power plant provides F-class fly ashes. This class of coal fly ash is normally produced from burning anthracite or bituminous coal and has pozzolanic properties.

The certified reference material BCR-176R from Institute for Reference Materials and Measurements (Geel, Belgium) was utilized for method validation. The material is a fly ash collected in the electrostatic filters of a municipal solid waste incineration (MSWI) plant. Prior to bottling, the fly ash was completely ground using a jet mill which rendered fine particles with a size distribution within 2−105 μm.

Analytical Protocol for Metal Leaching Studies. A weighed fly ash sample (250, 500, or 1000 mg) was transferred to a clean extraction chamber together with a small magnetic bar (1 cm long) and the overall components of the container were securely clamped. The extraction chamber was placed on a magnetic stirrer (P-Selecta Asincro, Spain) which was set at 1600 rpm. The Milli-Q water extractant is pumped continuously forward through the three extraction chambers at 4 mL min^{-1} using a single multisyringe pump (MP 1 in Figure 1) as precisely controlled by the user-friendly software and 5 (or 15 for BCR-176R) water leachate subfractions of 10 mL each per extraction chamber were collected. Thereafter, the second multisyringe (MP2) was activated to pump 0.11 mol L^{-1} acetic acid or 0.11 mol L^{-1} acetic acid/acetate buffer depending on the original pH of the fly ash[27] toward the three extraction chambers and 10 subfractions (or 30 for BCR-176R) amounting to a total of 100 (or 300) mL leachate per extraction chamber were now collected. The pH of overall leachates was measured, and 200 μL HNO_3 was added to each vial to prevent metal hydrolysis prior to analysis.

Total Dissolution of Sample and Dissolution of Residues. The residue leftover after extraction was collected and microwave digested aimed at conducting mass balance validation. The microwave digestion program is composed of four steps as recommended by the manufacturer,[28] yet the length of each step has been increased from 5 to 6 min. An identical procedure was utilized for determination of total concentration of trace metals in original coal fly ash. To this end, 0.1 g of the fly ash sample or residual solid fractions were transferred to PTFE vessels, and a mixture of 2.0 mL of fluoroboric acid, 2.0 mL of ortho-phosphoric acid, and 2.0 mL of nitric acid were added prior to microwave digestion (Milestone, model MLS-1200 Mega, Italy). Digests were finally diluted to 50 mL with Milli-Q water.

The overall subfractions and digested samples were analyzed by the Perkin-Elmer Optima 5300 DV inductively coupled plasma atomic emission spectrometer (ICP-AES). The U-6000AT$^+$ ultrasonic nebulizer (Perkin-Elmer, MA) was exploited for determination of low level concentrations of metals in leachates. A GemTip Cross-flow pneumatic nebulizer (Perkin-Elmer) was used for high level concentrations of metals in digested samples. The operating conditions for ICP-AES detection are given as follows: RF power, 1300 W; plasma Ar flow rate, 15 L min^{-1}; auxiliary Ar flow rate, 0.2 L min^{-1}; nebulizer flow rate, 0.5 L min^{-1}; rinse time, 60 s; sample flush time, 30 s; sample uptake delay, 60 s; read time, 5 s; view mode, axial; readout, peak height; and analytical wavelengths, 214.440 and 228.802 nm for Cd, 324.752 and 327.393 nm for Cu, 314.476 and 231.604 nm for Ni, 220.353 and 283.306 nm for Pb, and 206.200 and 202.548 nm for Zn.

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RESULTS AND DISCUSSION

Design of Multisyringe Flow Based Extractor. The overall stirred-flow chamber systems developed so far for performing operational chemical speciation of trace elements in soil/sediment substrates by sequential extraction[23,24,29−32] are semiautomatic analyzers capitalizing on continuous flow analysis. The extractants are replaced manually and propelled forward into the extractor by either negative or positive pressure using a peristaltic pump. If the extractor is not properly sealed, air is readily introduced into the extractor leading to unreliable results as a consequence of the disturbance of the solid/liquid extraction process. Further, the flow-setup is reported to lack ruggedness because of a low tolerance of fine particles that clog the chamber filter.[23,24]

To tackle the above drawbacks, a software-controlled flow-through extractor based on programmable flow is devised in this work. The Achilles' Heel of conventional continuous flow stirred chambers, that is, the peristaltic pump, is replaced with high-precision syringe pumps. By appropriate coupling of a given set of liquid drivers, sequential extraction schemes could be executed fully automatically in a parallel configuration as depicted in Figure 1 aimed at routine risk assessment of fly ash disposal or reuse.

Two-way ANOVA without replication[33] was herein applied to ascertain whether the slight differences in construction of extraction chambers have significant influence on trace element leachability in coal fly ash and to compare this variance with the day-to-day variation. The evaluation of F-test results revealed that there is no significant difference between these two variances and the random error of measurement, viz., residual mean squares, at a significance level of 0.05 for the suite of investigated metals, yet the interchamber variance is for most of trace elements assayed fairly higher than the interday variance (see Supporting Information table).

Leaching Test. The leaching behavior of trace metals in water-soluble and acid-soluble fractions of fractionation tests for fly ash should be interpreted in terms of solubility equilibria and leachate pH which determines the correlation between nominal phases and those actually released under given experimental conditions.

Water-Soluble Fraction. When distilled water is used as an extractant, metals loosely bound to solid particles are leached. However, the release of the water soluble fraction in fly ash samples is offset by the intrinsic buffer capacity of the water leachates at pH > 9 because of gradual dissolution of aluminum and calcium oxides as well as hydrolysis of aluminosilicates.[34−36] In batchwise single-equilibrium methods, the leachate pH increases gradually with time[34] and thus the water soluble pool of trace elements to be encountered in disposal sites or real scenarios, where water percolation through an ash profile should

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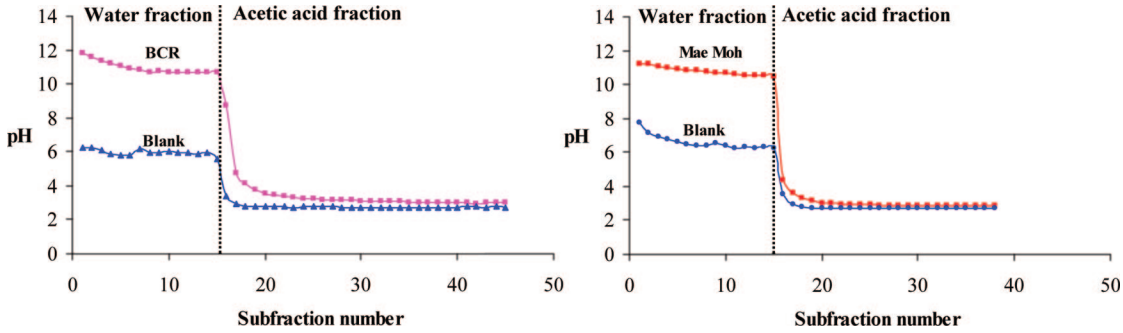

Figure 2. Variation of leachate pH for both CRM and real fly ash sample throughout dynamic fractionation tests using mild extractants. The results are the mean of three replicates. Relative standard deviations are <2%. Extraction time: 2.5 min per subfraction.

be accounted for, would be underestimated. On the contrary, leachate pH in dynamic methods decreases with time (see Figure 2) as a result of the multistage extraction nature of the flow-through leaching test and lower contact time between extractant and sample. The maximum release of Cu, Pb, Cd, and Zn actually occurs when leachate pH decreases from 11.8 to 10.6. Notwithstanding the fact that the water extractable content of the above trace elements in certified reference material (CRM) and real fly ash sample represents less than 0.5% of the total content, which is in well agreement with earlier reports on fly ashes,[37,38] the proposed flow-through method fosters accurate determination of this ecotoxicologically relevant fraction as dictated by the European Norm EN 12457 or German DIN 38414-S4 to assess eventual disposal or reuse of solid wastes.

Further, the multiple stirred-flow extractors could be coupled at-line to ICP-AES for simultaneous and real time monitoring of the extraction processes of the various samples and fast detection of extraction completion regardless of ash composition and aging. In batchwise methods, labor and time-consuming protocols are needed for accurate ascertainment of the leaching time for each particular ash sample as pinpointed by Derie.[34]

Exchangeable and Acid-Soluble Fraction. The TCLP leaching test was selected for evaluation of the overall pools of exchangeable and acid-soluble fractions of trace elements. It should be noted that this leaching test recommends the use of two mild extraction fluids for the same nominal target phases as a function of the alkalinity of the solid waste. To this end, a fly ash suspension with a liquid (water) to solid (L/S) ratio of 20 was prepared and pH was measured after acidification with 3.5 mL of 1.0 mol L^{-1} HCl. Following the endorsed criteria, fly ashes with high buffering capacity such as BCR-176R (pH of 10.2 ± 0.1 was obtained after acid addition) should be extracted with 0.1 mol L^{-1} acetic acid, and for those of low alkalinity such as the real coal fly ash sample (pH of 3.8 ± 0.1 after acidification), the extraction reagent should be changed to 0.11 mol L^{-1} acetic acid/acetate buffer (pH 4.9). However, when dynamic leaching was applied to the above real sample, no significant differences at the 0.05 significance level were found for the cumulative amounts of leached Cu, Cd, Pb, and Zn using either acetic acid or acetic acid/acetate buffer (see results in Table 1). This is because, as opposed to operationally defined equilibrium-based methods, accurate quantification of target fractions with no metal refixation effects is to be accomplished by the use of dynamic extractions.[14] In order to prevent potential spectral and nonspectral interferences in ICP-AES leachate analyses,[39] as a consequence of high concentration of dissolved salts in the buffer solution, 0.11 mol L^{-1} acetic acid is thus utilized as a TCLP reagent for the remainder of the work.

Table 1. Comparison of Leached Concentrations of Trace Elements in Coal Fly Ash Using Either 0.11 mol L^{-1} Acetic Acid or 0.11 mol L^{-1} Acetic Acid/Acetate Buffer (pH 4.9) in a Multisyringe Stirred-Flow Extraction Mode[a]

	concentration, $\mu g\ g^{-1}$	
elements	acetic acid	acetic acid/acetate buffer
Cu	9.5 ± 0.2	9.4 ± 0.2
Cd	0.11 ± 0.01	0.14 ± 0.02
Ni	0.46 ± 0.01	0.51 ± 0.03
Pb	0.34 ± 0.09	0.35 ± 0.02
Zn	2.6 ± 0.2	2.5 ± 0.2

[a] Results are given as the mean of three replicates ± standard deviation.

One of the most severe limitations of batchwise fractionation steps involving acetic acid as a leaching reagent is the difficulty in ensuring a complete extraction of metal species bound to carbonates. In fact, Huang et al.[7] have recently reported that the actual pH of leachates for BCR-176R and real fly ash sample in conventional leaching tests using 0.11 mol L^{-1} acetic acid/sodium acetate (pH 4.9) merely reached a value of 7.5 after 3 h of shaking. On the contrary, dynamic leaching involves a continuous solid/liquid equilibrium shift, whereby leachate pH gradually decreases until reaching the nominal pH of the extractant regardless of the alkalinity of the fly ash. Despite the high buffer capacity of BCR-176R, extraction at the actual pH of 2.9 is attained after 40 min extraction, as shown in Figure 2.

Figure 3 depicts the extractograms of Cu, Ni, Cd, Pb, and Zn in BCR-176R fly ash as obtained by the stirred-flow chamber dynamic sequential extraction method. Fresh extractant is continuously delivered to the extraction chamber until the metal in the target phase is completely leached as seen from signal gradually leveling off to baseline level, thereby simulating the worst-case field leaching scenarios as demanded in environmental surveillance programs.

Effect of Solid-to-Extractant Volume Ratio. It is reported in the literature[40] that a decreased solid to extractant (S/E) ratio

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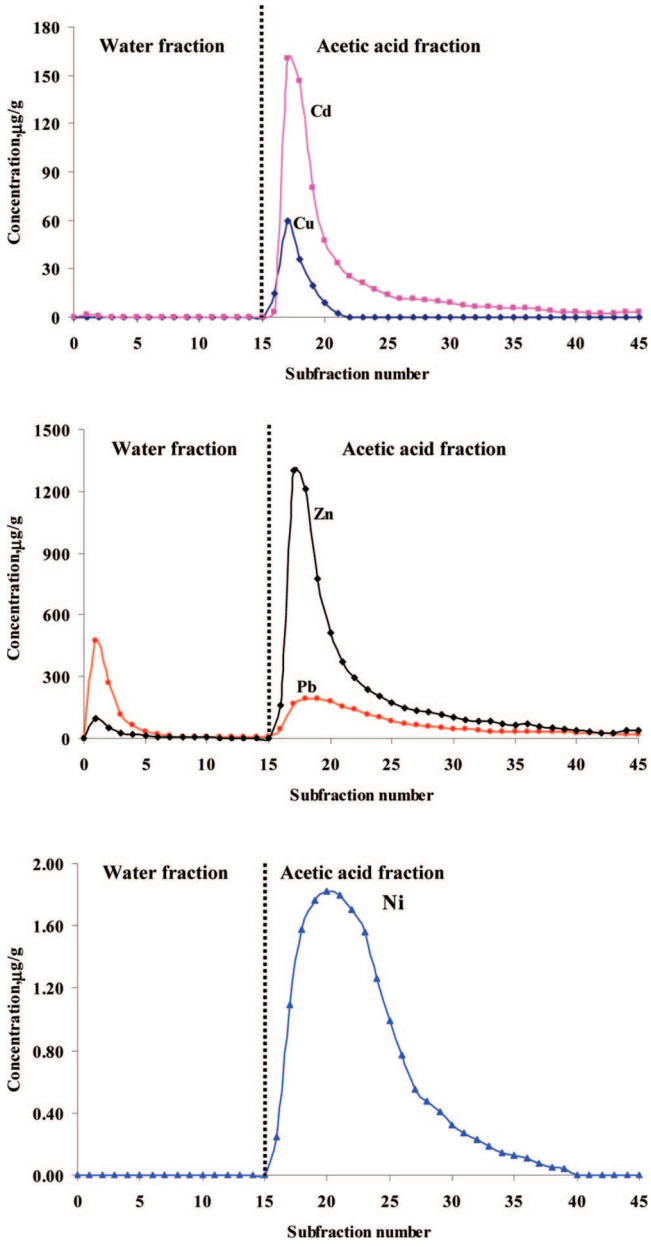

Figure 3. Averaged extractograms of trace elements in BCR-176R MSWI fly ash using a two-step dynamic sequential extraction and detection by ICP-AES.

in batchwise leaching tests generally increases the amount of trace metals in readily mobilizable fractions, which means that the S/E ratio needs to be carefully optimized in order to receive accurate information as to the maximum amount of soluble metal species.

As to dynamic sequential extraction in the stirred-flow chamber mode, the ANOVA results revealed that the decrease of S/FCV (free chamber volume) ratio from 1/10 to 1/40 for real fly ash did not significantly influence the leachability of trace elements at the 95% confidence level. Minimum deviations (<10%) and excellent repeatabilities (<2.5%) were obtained for the suite of determined elements (Cu, Zn, Ni, Pb, and Cd) at the various S/FCV ratios (see bar graphs within Figure A in the Supporting Information). As opposed to batchwise methods where the solubility equilibrium is most likely the limiting factor for trace element leachability, the optimization of the S/FCV ratio in dynamic leaching is not needed because extraction equilibria are shifted to the liquid phase until completion. This is in good agreement with earlier results by Shiowatana et al.[23] using a peristaltic pump for extractant delivery. However, these authors reported that S/FCV ratios as high as 1/10 for a chamber of 10 mL could not be assayed because of increased backpressure within the flow system.

Effect of Flow Rate. The most severe pitfall of continuous-flow stirred-chamber extractors furnished with peristaltic pumps is the instability of the flow rate of extractants over the sequential extraction protocols as a consequence of the gradual blockage of the membrane filter by solid particles.[24] It has been actually reported that the extractant flow rate for the acid-soluble fraction of trace metals dropped from 3.8 to 2.4 mL min^{-1} at an S/FCV ratio of 1/20.[23] To overcome this limitation, a more powerful constant-flow syringe pump that allowed trouble-free handling of large amounts of fly ashes and tolerated pressures of <7 bar was employed in this work. Under these conditions, nominal flow rates up to 8 mL min^{-1} were proven steady throughout the two-step sequential extraction scheme with maximum relative standard deviations of merely 0.8%.

The effect of flow rate on extractability of Cu, Cd, Ni, Pb, and Zn in real fly ash sample using the proposed multisyringe stirred-flow extractor was investigated over the range 2.0−8.0 mL min^{-1} as shown in Figure B in the Supporting Information. The concentration of target elements is calculated by summation of the leached amounts in each subfraction of the two-step sequential extraction scheme. No significant differences in extractability between 2.0 and 4.0 mL min^{-1} were encountered for any metal species. It should be noted that metals are not equally distributed between subfractions at the two different flow rates, yet the overall concentrations remain unaltered because quantitative leaching is achieved within the first few subfractions.

As to flow rates ≥6.0 mL min^{-1}, incomplete leaching of the solid waste was detected (see Figure B, Supporting Information) as a consequence of the formation of a heterogeneous suspension of the bulk sample within the flow chamber. Decreased contact times between the ash and extractant and dilution effects should be taken into account when affixing high flow rates for dynamic leaching. Moreover, if high flow rates are employed and the extraction kinetics of a given element is slow, the number of fractions collected for a given extractant should be increased to receive the total extractable fraction. The clear decrease in the leachability of Ni (see Figure B in Supporting Information), which is a poorly available element in fly ashes, might be likely explained by this fact. Accordingly, the flow rate for both water and acetic acid was set to 4.0 mL min^{-1} for further investigations.

Method Validation. The automated flow-based dynamic extraction method was applied to partitioning of various trace elements (Cu, Zn, Cd, Ni, and Pb) among readily mobilizable (the so-called water-soluble and acetic acid leachable) and fixed (residue) fractions in a real coal fly ash sample and BCR-176R MSWI fly ash. Experimental results along with total metal concentrations determined by microwave digestion and certified metal concentrations in BCR-176R are listed in Table 2. Accuracy and repeatability of fractionation analyses are thus critically evaluated. Coefficients of variation for extractable concentrations of Cu, Zn, Cd, Ni, and Pb using mild extractants are 0.9%, 2.3%, 9.1%, 2.2%, and 2.9%, respectively, for coal fly ash and 0.9%, 0.9%,

Table 2. Extractable Amounts of Trace Elements in Coal Fly Ash and BCR-176R Using a Two-Step Sequential Extraction Scheme in a Multisyringe Stirred-Flow Chamber Extraction Mode[a]

		concentration of metal found (μg g^{-1})									
element	sample	step I	step II	residue	total (mass balance)[b]	total dissolution (microwave digestion)	certified value[c] (μg g^{-1})	TCLP limits[d] (μg g^{-1})	recovery (%)[e]	t-stat $P = 0.05$	t-critical $P = 0.05$
Cu	coal fly ash	<LOD	9.28 ± 0.08	20.14 ± 0.03	29.42 ± 0.09	31.8 ± 1.5			92.5	2.99	3.18
	BCR-176R	5.22 ± 0.08	670 ± 6	317 ± 3	992 ± 7	1043 ± 6	1050 ± 35	25	95.1	2.14	4.30
Cd	coal fly ash	<LOD	0.11 ± 0.01	5.57 ± 0.11	5.68 ± 0.11	5.90 ± 0.13			96.3	2.39	2.57
	BCR-176R	0.67 ± 0.09	146 ± 4	62.2 ± 0.2	209 ± 4	212.9 ± 0.3	226 ± 10	1.0	98.1	2.15	4.30
Ni	coal fly ash	<LOD	0.45 ± 0.01	51.95 ± 0.11	52.40 ± 0.11	53.2 ± 0.8			98.5	2.12	3.18
	BCR-176R	<LOD	17.0 ± 0.6	86.5 ± 1.3	103.5 ± 1.4	112 ± 5	117 ± 3	20.0	92.4	2.11	4.30
Pb	coal fly ash	<LOD	0.34 ± 0.01	92.1 ± 0.4	92.4 ± 0.4	93.1 ± 1.6			99.2	0.74	2.57
	BCR-176R	1059 ± 50	1995 ± 83	1467 ± 7	4521 ± 97	4604 ± 40	5000 ± 250	5.0	98.2	1.69	2.57
Zn	coal fly ash	<LOD	2.61 ± 0.06	113.9 ± 0.2	116.5 ± 0.2	118 ± 3			98.7	3.17	3.18
	BCR-176R	269 ± 21	6728 ± 56	8725 ± 9	15722 ± 60	16033 ± 113	16800 ± 200	250.0	98.1	2.21	2.57

[a] Results are expressed as the mean of three fractionation analyses ± standard deviation. LOD: detection limit. [b] Summation of leachable concentrations in steps I and II plus residual concentration. [c] Certified concentrations are given as mean ± standard deviation. [d] EPA-TCLP (SW-846 method 1311).[27] [e] Calculated as the ratio between mass balance and microwave digestion.

2.7%, 3.5%, and respectively, for BCR-176R. Repeatabilities are much better than those reported for batchwise fractionation of BCR-176R which amounted to >14% RSD for Pb and Ni.[7] Method accuracy is ascertained by summation of extractable concentrations plus residue for each metal species and comparison (mass balance) with certified values or total concentrations estimated by microwave digestion. A good agreement between the sum of fractions and total (or certified) concentrations with recoveries ≥92% are attained for the suite of investigated elements in both samples analyzed. In fact, the t-test comparison of means confirmed the inexistence of significant differences at the 95% level for the overall fractionation analyses. As a consequence, the multisyringe stirred-flow chamber method is free from both additive and multiplicative matrix interferences, making the application of the method of standard additions unnecessary.

A relevant asset of the proposed assembly for two-step fractionation analyses is the likelihood of reducing leaching times from 42−48 h in equilibrium based methods to merely 37.5 min per 3 samples in moderately contaminated ash samples (e.g., Mae Moh coal fly ash) or 112.5 min per 3 samples in highly contaminated ashes (e.g., BCR-176R) which far exceeded the regulatory limits imposed by TCLP (see Table 2) for potential reuse or disposal of solid wastes.

The extractable amounts of trace elements in BCR-176R as obtained by use of mild extractants in multisyringe stirred-flow extraction were compared with batchwise sequential extraction results reported by Huang et al.[7] (see Table 3). Despite the different operational nature of both leaching tests, a good agreement was found for total readily mobilizable fractions of Cu, Pb, and Zn. For poorly available elements, such as Ni, stirred-flow acetic acid extraction leads to higher extractability. This is because dynamic methods, as opposed to equilibrium-based tests, are conducted at the nominal pH of the extractant. Notwithstanding the fact that dynamic methods are not prone to readsorption phenomena, an increased water-leachable cadmium concentration was found in the earlier batchwise extraction.[7] This is likely a consequence of the aging effect of the BCR solid waste which gives rise to enhanced immobilization of trace elements.

Table 3. Percentage Recoveries of Trace Elements in BCR-176R MSWI Fly Ash Following Dynamic Sequential Extraction with Mild Extractants and Comparison with Batchwise Extraction

elements	step I (%)	step II (%)	extractable metal (%)
Cu[b]	0.4	64.2	64.6
Cu[c]	3.0	58.0	61.0
Cd[b]	0.3	68.4	68.7
Cd[c]	26.0	50.0	76.0
Ni[b]	<LOD[a]	15.1	15.1
Ni[c]	<LOD[a]	3.0	3.0
Pb[b]	23.0	43.3	66.3
Pb[c]	4.0	60.0	64.0
Zn[b]	1.7	42.0	43.7
Zn[c]	3.0	40.0	43.0

[a] LOD: Limit of detection. [b] Results obtained in this work. [c] Results reported by Huang et al.[7] using batchwise extraction.

CONCLUSION

In this paper, a rugged, fully automated multisyringe-based stirred-flow chamber extraction system is proposed and validated for simultaneous fractionation analysis of three solid wastes as an expeditious alternative to dynamic flow-through microcolumn methods reported so far. The partitioning data of various trace elements among water-soluble acid-soluble fractions in coal and MSWI fly ashes obtained in this work is actually of prime importance in routine analysis aimed at ascertaining the eventual end use of solid wastes for construction purposes and soil amendment.

The flow-through extraction assembly devised is proven suitable for accurate assessment of the pool size of the most potentially mobile fractions of hazardous elements because it guarantees complete leaching of targeted phases without limitations due to the low solubility of the extractable phase in the solution medium or refixation of leached elements within the time frame of extraction. In contrast to conventional batchwise methods, there is no need for thorough optimization of the S/E ratio once sample representativeness is assured because of steady renewal of extractant medium. Most importantly, the extraction chamber admits larger sample amounts (≤1.0 g) as compared to a mere few micrograms (usually ≤50 mg) in flow injection/

sequential injection microcolumn approaches with no effect of leachant flow rate up to 6.0 mL min^{-1}. Therefore, the devised flow-through extractor is particularly suited for automatic fractionation of heterogeneous solid samples, such as industrial solid byproduct.

Current research work in our laboratory is aimed at exploiting the revised three-step BCR scheme for automated dynamic fractionation of four solids in parallel by use of multicommutation flow approaches in order to gain further knowledge as to metal solid phase associations in solid waste matrixes.

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NOTE ADDED AFTER ASAP PUBLICATION

The paper was posted on the Web on 8/22/08. The standard deviations for the "certified value" column were corrected. The paper was reposted on 9/30/08.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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CHAPTER 4

Critical evaluation of novel dynamic flow-through methods for automatic sequential BCR extraction of trace metals in fly ash

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ORIGINAL PAPER

Critical evaluation of novel dynamic flow-through methods for automatic sequential BCR extraction of trace metals in fly ash

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Abstract Two novel dynamic extraction approaches, the so-called sequential injection microcolumn extraction and sequential injection stirred-flow chamber extraction, based on the implementation of a sample-containing container as an external extraction reactor in a sequential injection network, are for the first time, optimized and critically appraised for fractionation assays. The three steps of the original Community Bureau of Reference (BCR) sequential extraction scheme have been performed in both automated dynamic fractionation systems to evaluate the extractability of Cr, Cu, Ni, Pb, and Zn in a standard reference material of coal fly ash (NIST 1633b). In order to find the experimental conditions with the greatest influence on metal leachability in dynamic BCR fractionation, a full-factorial design was applied, in which the solid sample weight (100–500 mg) and the extraction flow rate (3.0–6.0 mL min^{-1}) were selected as experimental factors. Identical cumulative extractabilities were found in both sequential injection (SI)-based methods for most of assayed trace elements regardless of the extraction conditions selected, revealing that both dynamic fractionation systems, as opposed to conventional steady-state BCR extraction, are not operationally defined within the selected range of experimental conditions. Besides, the proposed automated SI assemblies offer a significant saving of operational time with respect to classical BCR test, that is, 3.3 h versus 48 h, for complete fractionation with minimum analyst involvement.

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Keywords Dynamic fractionation · Sequential injection analysis · Stirred-flow chamber extraction · Microcolumn extraction · Coal fly ash · Trace elements

Introduction

There is a growing concern for accurate risk assessment of environmental pollutants, e.g., trace elements, in solid substrates such as soils, sediments, airborne particulate matter, and wastes as well. Yet, it is nowadays recognized that the potential hazardous of anthropogenic metal ions in contaminated soils, sediments, or solid wastes cannot be evaluated by measuring merely the total concentration of individual trace elements because the mobility, eventual bioavailability, and consequently, toxicity, largely depends on their chemical forms and types of metal solid-phase

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associations [1, 2, 3]. To this end, IUPAC defined the term "fractionation" as the identification and quantification of elements associated with predefined phases of solid compartments [4]. Batchwise sequential extraction methods are well-accepted operationally defined fractionation tools for environmental risk assessment of trace metals in solid matrices [5, 6] (e.g., sediments, soils, road and airborne particulates), but recently also in solid wastes, including sewage sludge [7], incinerator bottom and fly ash [8, 9], and coal fly ash [10] as well.

The Community Bureau of Reference (BCR) of the Commission of the European Communities (now termed Standards, Measurement and Testing Programme) launched in 1993 the "standard" BCR sequential extraction method [11]. The idea behind was to propose a harmonized batchwise protocol to fractionate metals into three fractions labeled according to the chemical processes involved, that is, (1) the exchangeable and water- and acid-soluble fraction, (2) the reducible fraction, and (3) the oxidizable fraction. The BCR scheme was originally proposed for partitioning of trace elements in soils and sediments [12], but later, it has proven suitable for other solid substrates [5] such as fly ash, which is always regarded as a hazardous waste owing to the high trace metal content and potential mobilization of toxic elements under environmentally changing conditions [13, 14]. Thus, fly ash has been selected in this work as a model solid waste sample to ascertain the leachability of trace metals exploiting the BCR procedure.

Classical sequential extraction protocols are however inadequate to get knowledge on the leaching kinetics of trace elements under the action of the various extractants, and most importantly, experimental results might be biased as a result of metal readsorption [2, 5, 6], that is, elements released by one extractant might associate with other undissolved solid components or freshly exposed surfaces within the time frame of the extraction step. Hence, the contents of trace metals bound to the nominal soil fraction for a given extractant would be underestimated, while the metal mobility in subsequent phases would be overestimated. Moreover, batch methods for well-buffered samples, e.g., solid wastes, are proven not to operate under nominal pH of extractants [15].

To overcome these limitations, an appealing alternative to batchwise sequential extraction methods consists of performing the leaching tests in a flow-through dynamic mode [5, 6, 16, 17]. In dynamic (non-equilibrium) extraction approaches, the solid sample under investigation is loaded into a suitable container, which is exposed continuously to fresh extractant volumes by resorting to flow-based approaches [18–27]. It should be also noted that environmental occurring processes are always dynamic, and thus, non-equilibrium based methods could better imitate real-life scenarios than the batchwise counterparts. The dynamic leaching behavior of solids is actually of great importance to mimic, for example, the soil-groundwater pathway of pollutants and percolation of rainwater through disposed solid wastes.

The aim of this work is to critically compare and evaluate the pros and cons of two recently proposed fully automated dynamic extraction systems, the so-called sequential injection microcolumn extraction (SI-MCE) [26, 28–30] and sequential injection stirred-flow chamber extraction (SI-SFCE) [31], capitalized on the liquid handling capabilities of the second generation of flow analysis, that is, sequential injection (SI) analysis [32], which has been proven more rugged for fractionation of solid samples than flow-injection/continuous-flow manifolds furnished with peristaltic pumps [16, 18, 22, 27]. The core of SI is a multiposition selection valve, the central port of which via a holding coil is connected to a high-precision bidirectional syringe pump operating as the liquid driver. Through the internal rotary conduit of the valve, the syringe pump can be made to address each of the ports of the valve furnished with the different extractants, which are sequentially exposed to the solid sample via flow reversal. The main difference between both systems relies on the nature of the extractor, the sample being contained either in a dedicated microcolumn or a stirred-flow chamber in SI-MCE and SI-SFCE, respectively. Both fractionation methods are fully computer-controlled and exploit the flow-programming features of SI for automation of the BCR original protocol in a dynamic fashion. It should be borne in mind that the BCR procedure was endorsed to harmonize extraction protocols and to facilitate direct comparison of results between studies and reported data [5], the latter being also aimed in this work.

Method intercomparison was effected by investigation of the extractability of Cr, Cu, Ni, Pb, and Zn in a standard reference material of coal fly ash (NIST 1633b), which was also utilized for validation purposes. A two-level full-factorial design is employed to ascertain which parameters have significant influence on metal extractability in both flow-through extraction assemblies. Two critical factors reported in batchwise fractionation, that is, the liquid to solid ratio and the extraction time, are evaluated by optimization of solid sample weight and extractant flow rate, respectively, for both dynamic extraction methods.

Experimental

Reagents, solutions, and standard reference material

All reagents were of analytical grade and Milli-Q water (Millipore Synthesis A10, France) was used throughout. A 0.11 mol L^{-1} acetic acid solution used as the first extractant in the BCR procedure for nominal release of water soluble and exchangeable ions, and those (co)precipitated with

carbonates was prepared by dilution of 6.3 mL of glacial acetic acid (99.8%, Scharlau, Barcelona, Spain) with water to 1 L. A 0.1 mol L^{-1} hydroxylammonium chloride solution used for leaching of metals bound to Fe and Mn oxyhydroxides was prepared by dissolving 6.9 g of hydroxylammonium chloride (99.5%, Panreac, Barcelona, Spain) in 985 mL of water, to which concentrated nitric acid was added until pH 2.0 prior to making up to 1 L. An 8.8 mol L^{-1} hydrogen peroxide solution utilized for extracting trace elements complexed with residual organic matter in the ash or precipitated as sulfides was prepared by mixing 900 mL of hydrogen peroxide stock solution (30% *w*/*w*, 1.15 g mL^{-1}, Scharlau) with 85 mL water followed by pH adjustment to 2.0 with concentrated nitric acid before making up to 1 L.

A multielement standard solution XI (AccuTrace Reference Standard, AccuStandard, New Haven, CT, USA) was employed for external calibration. Diluted working solutions were prepared daily using a matrix-matched procedure for analyses of acetic acid, hydroxylammonium, and hydrogen peroxide leachates. All glassware and polyethylene containers were previously soaked in 10% (*v*/*v*) HNO_3 and rinsed with deionized water prior to solution preparation.

A coal fly ash standard reference material (NIST 1633b) from the National Institute of Standard and Technology was used for comparative fractionation purposes and validation of the two dynamic extraction systems. The reference material is a bituminous coal fly ash, sieved through a nominal sieve opening of 90 μm, with certified total concentrations for a given number of elements.

Sequential injection manifolds for dynamic extraction

Both SI-fractionation setups (SI-MCE and SI-SFCE) comprised a 5,000-step bidirectional syringe pump (SP; Crison Instruments, Alella, Barcelona, Spain) for automatic handling of leaching reagents and delivery of well-controlled volumes to the solid sample as contained in a biconical microcolumn for SI-MCE (see Fig. 1, solid line A) or in stirred-flow chamber in SI-SFCE (see Fig. 1, dash line B), an eight-port multiposition selection valve (SV; Crison Instruments) for selection of appropriate BCR extractants, and a 45-position rack autosampler (Micro Sampler Crison) for collection of leachate subfractions.

The automatic SP was furnished with a 5-mL syringe (Hamilton, Switzerland) and a three-way solenoid valve at its head, which allowed connection with either the manifold or the carrier (water) reservoir. The central port of the SV was connected to SP via a holding coil (HC), which consisted of a 3.0-m-long polytetrafluoroethylene (PTFE) tubing (1.5 mm i.d.), with an approximate internal volume of 5.3 mL. The outlets of SV were connected to the extractant reservoirs, sample container, or waste through rigid PTFE tubing (1.5 mm i.d.) using polyetheretherketone (PEEK) fittings.

For instrumental control of SP, SV, and autosampler, the software package AutoAnalysis 5.0 (Sciware, Palma de Mallorca, Spain) was employed. The custom-built software based on dynamic link libraries (DLLs) is composed of a single and versatile main protocol, to which individual DLLs for control of the instrumentation were attached [33].

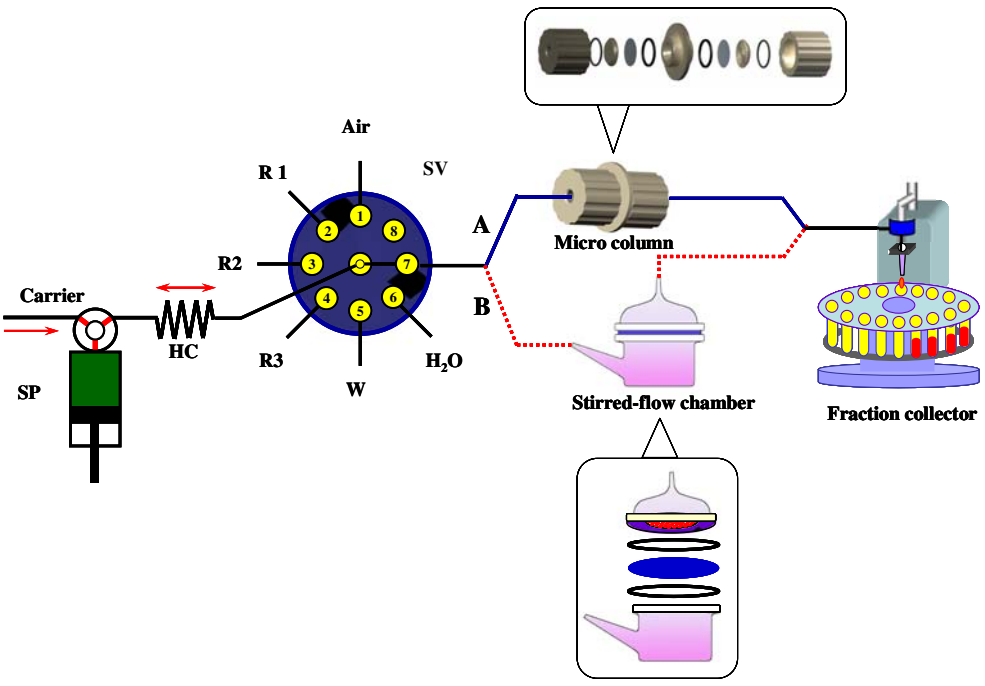


Fig. 1 Schematic illustration of the SI manifolds devised for dynamic BCR fractionation of trace metals in fly ash using either a biconical microcolumn (*solid line A*) or stirred-flow chamber (*dash line B*) as sample container. *R1* 0.11 mol L^{-1} CH_3COOH, *R2* 0.1 mol L^{-1} $NH_2OH{\cdot}HCl$ at pH 2, *R3* 8.8 mol L^{-1} H_2O_2 at pH 2, *SP* syringe pump, *HC* holding coil, *SV* selection valve, *W* waste

The microcolumn employed in SI-MCE has been described in detail elsewhere [24]. It was made of PEEK and comprised a central dual biconical shape sample container. The column was equipped with filters and filter supports and caps at both ends. Membrane filters (Fluoropore TM, Millipore, 13 mm diameter) with 0.45 μm pore size were used in this work for retention of the fly ash. A maximum sample amount of 200 mg NIST 1633b could be packed without observing undue flow backpressure.

The stirred-flow chamber in SI-SFCE was constructed from borosilicate glass to have a capacity of ca. 10 mL, as detailed in earlier publications [18, 27]. A rubber gasket was placed on top of the chamber followed by a nylon filter (GE Osmonics Labstore, MN, USA) of 0.45 μm pore size and 47 mm diameter to allow dissolved matter to flow through but retain ash particles. The setup was completed with a second rubber gasket and the cover on top of the stirred-flow chamber. The inlet of the chamber was connected to SV while the outlet to the autosampler using small pieces of Tygon tube and PTFE tubing of 1.5 mm i.d. A weighed fly ash sample (200, 350, or 500 mg) was transferred to the stirred-flow chamber together with a small magnetic bar (1 cm long), and the overall components of the container were securely clamped. The chamber was placed on a magnetic stirrer (P-Selecta Asincro, Spain), which was set at 1,600 rpm.

Detection instrument

The concentrations of trace elements in leachates, residual fractions, and original sample following microwave digestion were determined with the Perkin-Elmer Optima 5300 DV inductively coupled plasma optical emission spectrometer (ICP OES). The U-6000AT^{+} ultrasonic nebulizer (Perkin Elmer, MA, USA) was exploited for determination of low level concentrations of trace elements in leachates. A GemTip Cross-flow pneumatic nebulizer (Perkin Elmer) was however employed for analyses of residual fractions and fly ash because of the high level concentrations of dissolved elements. The operating conditions for ICP OES detection are given as follows: RF power, 1,300 W; plasma Ar flow rate, 15 L min^{-1}; auxiliary Ar flow rate, 0.2 L min^{-1}; nebulizer flow rate, 0.5 L min^{-1}; rinse time, 60 s; sample flush time, 30 s; sample uptake delay, 60 s; read time, 5 s; view mode, axial; readout, peak height; and analytical wavelengths, 205.560 and 267.716 nm for Cr, 324.752 and 327.393 nm for Cu, 232.003 and 231.604 nm for Ni, 220.353 and 283.306 nm for Pb, and 206.200 and 202.548 nm for Zn. Detection (LOD) and quantification (LOQ) limits for the suite of investigated elements were calculated as $3\sigma_{blank}$ and $10\sigma_{blank}$, respectively, for each extraction reagent (see Table 1).

Analytical procedures

Before initialization of the dynamic sequential extraction protocols, the biconical microcolumn and stirred-flow chamber in SI-MCE and SI-SFCE systems, respectively, were filled with a given amount of fly ash, which was selected attending the internal volume of the sample containers.

Both fully automated analytical procedures started with the aspiration of a 150-μL air plug from port 1 of SV into HC. The role of the air segment was to prevent dispersion of the leaching reagent into the carrier solution. Afterward, SP was set to aspirate 5.0 mL of 0.11 mol L^{-1} acetic acid extractant from port 2 into HC at a flow rate of 10.0 mL min^{-1}. The entire extractant plug was then dispensed backward to port 7 at the affixed extractant flow rate, namely, 3.0, 4.5, or 6.0 mL min^{-1}, according to the optimization procedure as detailed below. In this step, the extractant was pumped through either the microcolumn extractor (see Fig. 1, solid line A) or the stirred-flow chamber extractor (see Fig. 1, dash line B), thereby allowing the release of the acid-soluble fraction to take place. For each two cycle runs, the extracts from the extractor devices were collected in a single plastic vessel, thus totally amounting to 10 mL. The above analytical protocol was repeated 15 times for both flow-through systems, whereby 150 mL of acid-soluble fraction was collected. Before commencing with the next extraction, a washing step was implemented by aspiration of 5.0 mL of

Table 1 Detection and quantification limits for trace elements in BCR extraction media as determined by ICP OES with ultrasonic nebulizer

Extraction reagent	Cr		Cu		Ni		Zn		Pb	
	LOD (μg L^{-1})	LOQ (μg L^{-1})	LOD (μg L^{-1})	LOQ (μg L^{-1})	LOD (μg L^{-1})	LOQ (μg L^{-1})	LOD (μg L^{-1})	LOQ (μg L^{-1})	LOD (μg L^{-1})	LOQ (μg L^{-1})
Acetic acid	0.06	0.19	0.08	0.28	0.12	0.42	0.17	0.55	0.63	2.11
Hydroxyl-ammonium	0.06	0.21	0.66	2.20	0.18	0.60	0.16	0.54	0.28	0.94
Hydrogen peroxide	0.36	1.19	0.19	0.64	0.15	0.51	0.49	1.63	0.63	2.10

Analytical wavelengths (nm): 205.56 (Cr); 324.752 (Cu); 231.604 (Ni); 202.548 (Zn) and 220.353 (Pb)

Milli-Q water from port 6 into HC, whereupon it was delivered to the extraction devices and collected in a new plastic vial. A single cleaning step was required for SI-MCE, as opposed to SI-SFCE, which needed two consecutive rinses because of increased chamber volume. The amount of metals leached in the cleansing step was summed to the content of the subfractions collected in the former extraction. Thereafter, the 0.1 mol L^{-1} hydroxylammonium chloride extractant was automatically aspirated from port 3 following a similar sequence as described above for acetic acid, and 15 subfractions (150 mL of reducible fraction) were collected for both SI-MCE and SI-SFCE. A second washing step with Milli-Q water was then performed to prevent zone overlapping between the reducing and oxidizable reagents.

Prior to the aspiration of the 8.8 mol L^{-1} hydrogen peroxide extractant, both the ash containing extraction devices and the hydrogen peroxide extractant reservoir were placed in a thermostated bath to assure that this extraction step occurs at 85±2 °C as endorsed in the BCR method, whereupon the oxidizing extractant was aspirated from port 4, and ten subfractions (100 mL of oxidizable fraction) were collected for both SI-based extraction systems. Afterward, a third washing step was implemented before collection of residue for determination of the pool of less mobile trace elements.

Dissolution of residues and determination of total concentration of metals

The residue leftover after extraction was collected and digested in a closed-vessel microwave digestion system (MLS-1200 Mega) from Milestone (Sorisole, Italy) aimed at conducting mass balance validation. The microwave digestion program was composed of five consecutive steps as specified by manufacturer [34], yet the length of each step has been increased from 5 to 7 min to ensure complete dissolution. The program consisted of a first step at a power of 250 W, followed by 400, 650, and 250 W and a last stage without power supplied. An identical program was utilized for determination of total concentration of trace metals in NIST 1633b. Hence, an amount of 0.1 g of NIST 1633b (or residual fraction) was transferred to PTFE vessels and a mixture of 2.0 mL fluoroboric acid, 2.0 mL orthophosphoric acid, and 2.0 mL nitric acid was added prior to microwave digestion. Digests were finally diluted to 50 mL with Milli-Q water.

Two-level factorial design

In a two-level full-factorial experimental design, a well-defined number of experiments is conducted for facilitating the optimum experimental conditions to be obtained [35, 36]. The design was utilized in this work for ascertaining whether the sample weight and extractant flow rate have a significant effect on the leachability of trace elements in dynamic SI-MCE or SI-SFCE. The relevance of the interaction between these two factors was evaluated as well. The analysis of response data (cumulative leachability in BCR) was performed in a dimensionless coordinate system using factor coding [37]. In this factor space, the highest and lowest levels are given as +1 and −1, respectively. The involved coded and uncoded levels for each factor are presented in Table 2. Three replicates of the center of the design (center point) were also included in the design to ensure that the variability found is on account of the factor effect in lieu of random error. Notwithstanding the fact that the replicate measurements were only conducted for the center point, the calculated uncertainty was utilized as an estimate of the variability for the entire experimental domain.

The statistical computer package StatGraphics (StatGraphics Centurion XV, Stat Point, Herndon, VA, USA, 2005) was used to build the two-level factorial design with a total number of seven runs, including center points. The effects of individual factors and their second order interactions were thus investigated.

Results and discussion

Strengths and weakness of SI-MCE and SI-SFCE systems

Both automated sequential extraction assemblies have pros and cons, which are a result of each particular configuration and chamber design. It should be noted that the sample containers differ significantly as to the internal volume, which determines the free chamber volume (FCV). The volume capacity of the stirred-flow chamber is 10 mL [18], while the internal volume of the biconical microcolumn in SI-MCE is merely 750 μL [26]. As a consequence of decreased FCV, the subfractions of leached trace elements were in SI-MCE less diluted, thereby fostering a more accurate quantitation of mobile pools. Further, the overlapping of fractions on changing of extractants was kept to minimum in SI-MCE. Yet, the major shortcoming of the microcolumn extractor as compared to the stirred-flow chamber is the amount of fly ash that can be handled without increase of flow impedance. In fact, a maximum solid amount of 300 mg has been partitioned in SI-MCE [26,28], whereas stirred-flow chambers admitted solid samples up to 5 g [38, 39]. Hence, highly heterogeneous samples, such as solid wastes, should be better handled in SI-SFCE because sample representativeness might not be assured in SI-MCE. Yet, another advantage of SI-SFCE is the use of magnetic stirring, thereby assuring an intimate

contact between the solid and extractant, and thus, an increased effective surface area of solid particles for leaching. On the contrary, preferential flow channels for the extractant might be eventually originated in SI-MCE as a consequence of the progressive tighter packing of solid material within the flow-through microcolumn, which would hence lead to the underestimation of the content of trace elements bound to a given mineralogical phase.

Dynamic flow-through extraction/fractionation tests capitalized on continuous-flow or flow-injection analysis have been earlier reported in the literature [18–23, 25, 27]. Regular recalibration of the peristaltic pump used for delivery of extractant solutions or frequent renewal of flexible Tygon pump tubing was yet imperative for routine analyses [16, 18, 20, 25, 27]. On the other hand, minimum operational maintenance was needed in both SI-MCE and SI-SFCE systems because of the exploitation of a rugged, bidirectional syringe pump as a liquid driver and the fully mechanized control of physical variables and extractant changeover by user-friendly software. Both SI setups were actually capable of enduring the flow backpressure caused by fine particle clogging of membrane filters in the extractors whereby assuring steady extractant flow rates. Furthermore, taking advantage of the versatility of the syringe pump, four operational modes for handling leaching solutions (i.e., unidirectional flow, bidirectional flow, multi-bidirectional flow, and stopped flow) might be readily applied on the basis of the leaching kinetics of target elements, with no need for manifold reconfiguration [16, 30]. Despite the discontinuous operational nature of SI systems, on-line hyphenation of flow-through extractors with atomic absorption/emission spectrometers has been yet proven feasible via the usage of appropriate interfaces to facilitate real-time monitoring of the extraction process [28, 29].

SI-MCE and SI-SFCE setups have however some disadvantages related to the implementation of filter membranes in the flow manifolds for retaining the solid substrates (e.g., fly ashes) because membrane pore sizes should be carefully selected attending both particle size and packed solid amount to prevent increased flow resistance and also the need for specific instrumentation and software. In case of SI-SFCE, paramagnetic metals should not be monitored as a consequence of the use of magnetic stirring.

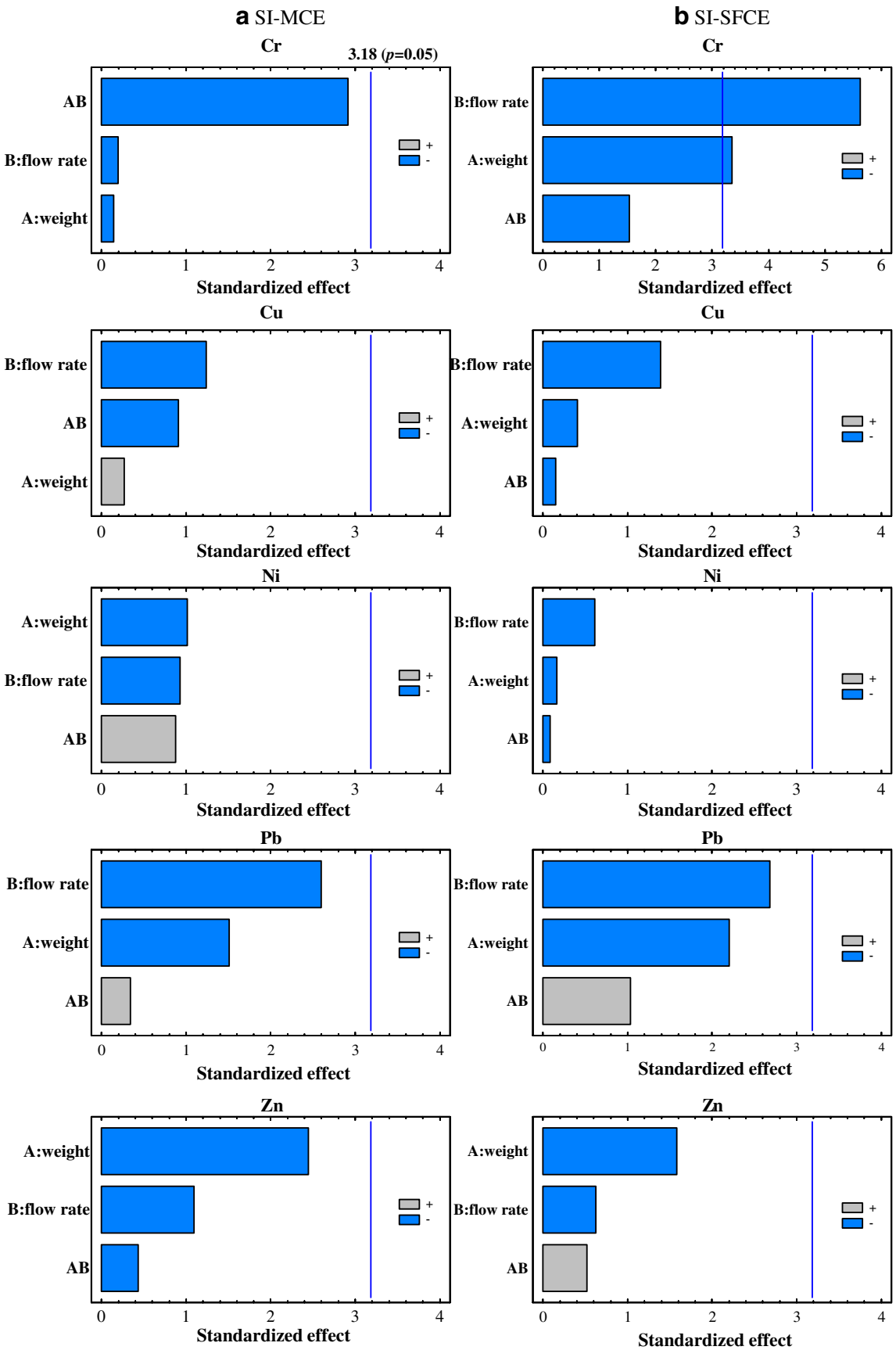
Fig. 2 Pareto charts of standardized effects (p=95%) for the comparison of metal extractability in NIST 1633b as performed by SI-MCE and SI-SFCE using BCR leaching test. **a** SI-MCE, **b** SI-SFCE

Optimization of sequential injection fractionation procedures

The exploration of experimental variables on metal leachability in both dynamic fractionation procedures was performed by resorting to a full-factorial design at two levels with three replicates of the center point. Solid sample weight and extractant flow rate were selected as factors, and the metal extractability given as the summation of extractable amounts of a given metal in the three BCR steps was taken as analytical response. Sample mass ranged from 100 to 500 mg attending the capacity of the flow-through extractors and flow rates varied within the range of 3.0–6.0 mL min^{-1} according to previous results in SI-based fractionation methods [24, 26, 30, 31]. Table 2 shows the

Table 2 Complete factorial design for evaluation of significant variables in metal leachability of NIST 1633b using SI-MCE and SI-SFCE

Run	Solid sample weight, g		Flow rate, mL min^{-1}		Metal extractability, mg kg^{-1}				
	Uncoded	Coded	Uncoded	Coded	Cr	Cu	Ni	Pb	Zn
Sequential injection microcolumn extraction (SI-MCE)									
1	0.10	−1	6.0	+1	17.98	24.69	7.17	7.35	28.86
2	0.10	−1	3.0	−1	16.47	25.31	8.85	10.97	31.70
3	0.20	+1	6.0	+1	16.28	23.48	7.04	5.91	16.49
4	0.20	+1	3.0	−1	18.01	27.53	7.09	8.69	23.05
5	0.15	0	4.5	0	18.12	27.79	8.22	8.22	20.73
6	0.15	0	4.5	0	16.85	27.85	6.83	6.83	18.67
7	0.15	0	4.5	0	17.26	24.64	6.15	7.35	19.01
Sequential injection stirred-flow chamber extraction (SI-SFCE)									
1	0.20	−1	6.0	+1	16.19	23.43	5.80	7.06	21.33
2	0.20	−1	3.0	−1	17.72	26.56	6.08	6.08	22.53
3	0.50	+1	6.0	+1	14.37	22.04	5.67	6.65	20.22
4	0.50	+1	3.0	−1	17.04	25.91	6.04	6.04	20.33
5	0.35	0	4.5	0	16.30	22.23	6.14	7.06	20.19
6	0.35	0	4.5	0	15.76	21.90	5.97	6.73	21.68
7	0.35	0	4.5	0	15.81	20.06	6.92	6.90	19.47


a SI-MCE
b SI-SFCE
Cr
3.18 (p=0.05)
AB
B:flow rate
A:weight
Standardized effect
Cu
Ni
Pb
Zn
+
-

experimental design matrix where the highest and lowest values of each factor and the processed response for the suite of investigated metal species are compiled. The analysis of the results yielded Pareto charts of the main effects (see Fig. 2) that facilitate ascertainment of the relevance of two-factor interactions. Pareto charts are histograms where the length of each bar is proportional to the absolute value of the estimated effect, namely, fly ash weight (A), extractant flow rate (B), and interaction between them (AB), on metal extractability. The cross vertical line indicates the significance of each factor at the 95% confidence level, corresponding in our case to a Student's t of 3.18 for three degrees of freedom. An effect exceeding this vertical line should be regarded as statistically significant with regard to BCR metal extraction. The smaller the calculated p values for a given factor, the more significant are the corresponding coefficient terms on the response surface of the factorial design [35, 36, 40]. The positive (light) or negative (dark) bars denote those scenarios where the metal extractability is improved or reduced, respectively, when increasing the given factor from the lowest to the highest level.

Pareto charts for SI-MCE (see Fig. 2a) revealed that none of the factors and neither their interactions were statistically significant for BCR extractability of the suite of target metal ions in NIST 1633b ($p>0.05$) within the investigated range of experimental conditions. As opposed to batchwise leaching tests, the SI-MCE method is thus not operationally defined because no significant differences in extractability were obtained regardless of the extraction conditions selected. It was however observed that the interaction between extractant flow rate and fly ash weight was the most influential factor on Cr leachability, and therefore, a planar response surface might not accurately describe the system. For Cu and Pb, the lower the extractant flow rate, the higher was the cumulative metal extractability. On the contrary, fly ash weight was the most relevant factor on the release of Zn. These differences are attributed to the different mobility and extraction kinetics of the various target metals under the action of BCR extractants in a dynamic fashion.

As to SI-SFCE, the Pareto graphs (see Fig. 2b) demonstrated that both investigated factors and interactions were not influencing significantly the extractability of Cu, Ni, Pb, and Zn. Yet, the experimental p values of Cr in fly ash weight and extractant flow rate were 0.044 and 0.011, respectively. In contrast to SI-MCE, both factors were thus statistically significant at the 95% level. This is actually a consequence of the improved repeatability of SI-SFCE as compared to SI-MCE, thereby leading to a decreased random error. The extractant flow rate should be regarded as the most influential factor in SI-SFCE for Cr, Cu, Ni, and Pb, which is in good agreement with earlier observations [31]. The lower the flow rate, the higher was the extractability, as might be expected due to increased contact times between fly ash and extractants, improved mixing conditions and decreased dilution effects. For the highest flow rate, namely, 6.0 mL min^{-1}, the number of subfractions collected for dynamic BCR leaching should be increased to receive the total extractable fraction.

Because the standardized effects for most of the factors and interactions were below 3.18 ($p=0.05$) for the target metals within the range of experimental conditions tested, dynamic fractionation methods can be readily optimized using one-at-a-time approaches without risks of biased interpretation of fractionation data. For better comparison of SI-MCE with SI-SFCE and in order to set the basis for harmonization of dynamic flow-through sequential extraction schemes, the extractant flow rate and fly ash amount were affixed to 3.0 mL min^{-1} and 200 mg, respectively, for both SI systems. These automated extraction setups should be actually regarded as appealing alternatives to batchwise methods to overcome the poor recoveries encountered, e.g., in BCR, when downscaling the protocol to 100–200 mg sample amounts [41].

Leaching profiles

When comparing the dynamic fractionation data for the target metal species in NIST 1633b as obtained by SI-MCE and SI-SFCE, respectively, similar trends in leaching patterns (also called extractograms) were observed for the overall trace elements (see Fig. 3) excepting for Pb, as will be later explained. Each metal species was mostly leached in the first six subfractions of each BCR step, and the metal concentrations rapidly decreased to reach a steady level in the following leachate subfractions. Slow leaching was, however, detected for the oxidizable fraction of Ni and Zn in SI-MCE, which is attributed to the operation under quiescent (non-stirring) mixing conditions.

According to the extractograms illustrated in Fig. 3, the peak profiles in step II and III were in SI-MCE shifted to lower extractant volumes because the concentration gradient upon changing of BCR extractant was steeper than that of SI-SFCE**.** As can be observed in Fig. 4, the nominal pH of the extractants was rapidly reached in SI-MCE in merely one subfraction, yet a large number of subfractions (≥3) was needed in SI-SFCE for operating under nominal pH conditions as a result of the increased FCV, and hence, the extractant phase was renewed more slowly than in SI-MCE. It should be noted that one of the most severe shortcomings of batchwise steady-state leaching tests when applied to fly ash fractionation is the difficulty in ensuring extraction under nominal extractant pH due to the buffer capacity of aluminum and calcium oxides in the sample [15, 42]. This is however not a limitation in dynamic flow-through

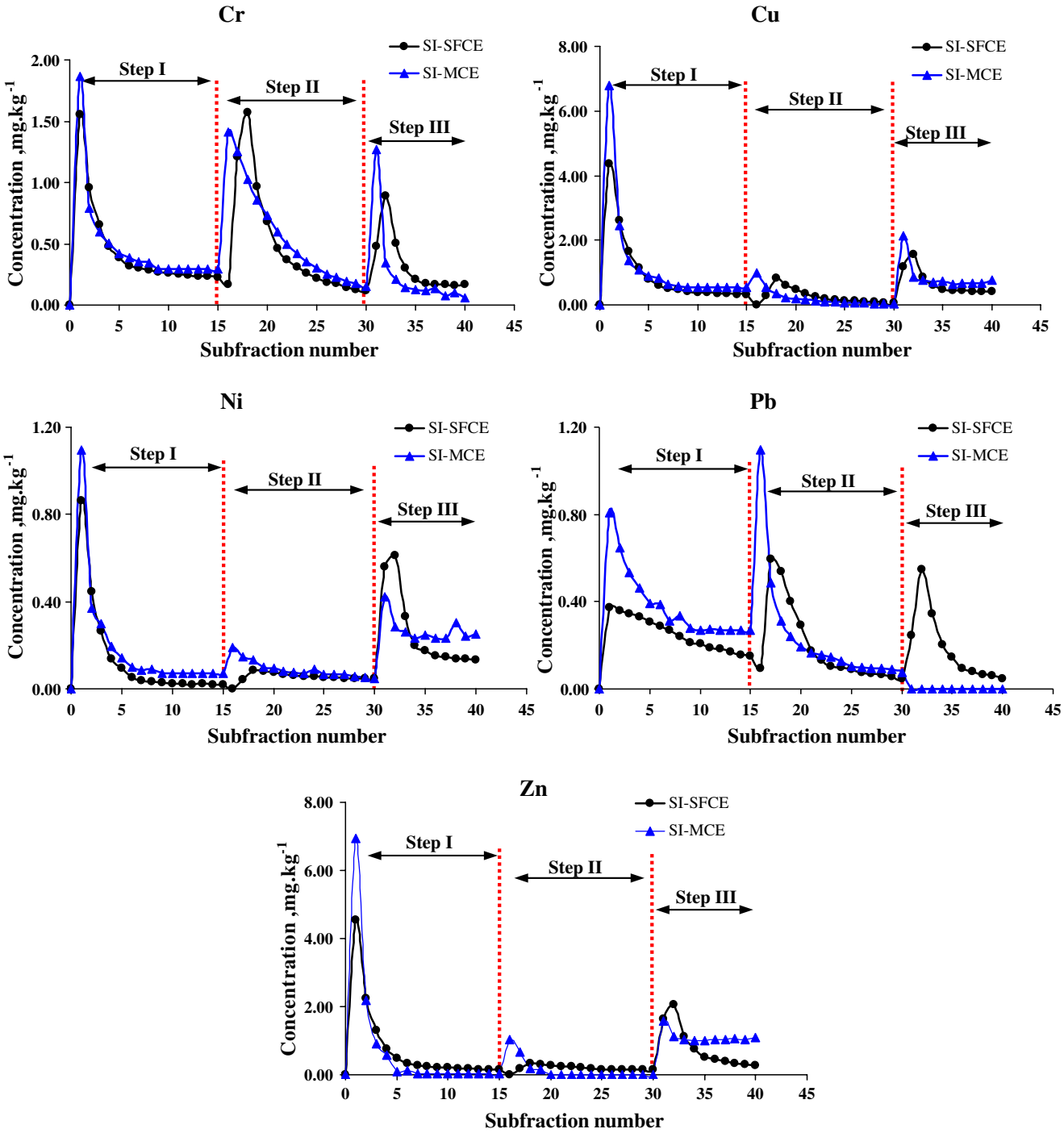


Fig. 3 Averaged extractograms of Cr, Cu, Ni, Pb, and Zn in NIST 1633b for dynamic BCR fractionation exploiting sequential injection microcolumn extraction (*SI-MCE*) and sequential injection stirred-flow chamber extraction (*SI-SFCE*)

fractionation because of the multistage nature of the extraction process, whereby leachate pH gradually reaches to the nominal extractant pH regardless of the alkalinity of the original fly ash.

Experimental data compiled in Fig. 3 revealed that Cu and Zn were the most sensitive elements to acidification processes inasmuch as the major concentration of these trace metals was released in the first step of the BCR protocol (55% and 44% of the total extracted element for Cu and Zn, respectively), whereby Cu and Zn were most likely bound to carbonates. On the contrary, Pb was mostly leached in step II of the sequential extraction scheme, which is in good compliance with previous researchers [28, 43]. Hence, Pb was mainly bound to manganese oxy-

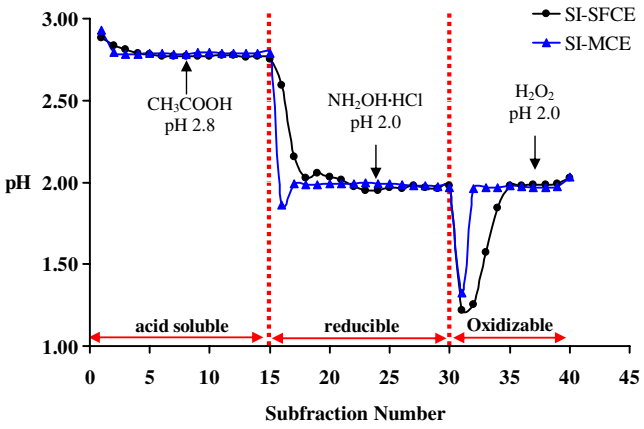


Fig. 4 Variation of leachate pH for dynamic BCR fractionation of NIST 1633b as obtained by exploiting sequential injection microcolumn extraction (*SI-MCE*) and sequential injection stirred-flow chamber extraction (*SI-SFCE*). Rinsing water subfractions are not included

hydroxides and amorphous iron oxides, that is, associated to easily or moderately reducible fractions.

Validation of the flow-through dynamic extraction methods and comparison with previously obtained extraction data

The fractionation data for trace elements in NIST 1633b as obtained by dynamic BCR extraction exploiting SI-MCE and SI-SFCE are presented in Table 3. In spite of the differences in chamber designs, the cumulative metal extractability in the three-step BCR test was for each individual element investigated in SI-MCE not significantly different than that of SI-SFCE at the 95% confidence level [44]. However, significant differences at the 0.05 level were found for Pb when comparing metal leachability in the acid-soluble and oxidizable fractions of SI-MCE with respect to those of SI-SFCE (see Table 3 and Fig. 3). SI-MCE yielded improved extractability for the most mobile fraction of Pb, that is, the acid-soluble fraction and decreased leaching for the poorly available oxidizable fraction. This effect is attributed to the contribution of the readsorption phenomenon for mobile forms of Pb in the acetic acid leachate of SI-SFCE because of the improved contact time between fly ash and acetic acid per extractant volume, which is the result of the larger FCV of the stirred-flow container as compared to the biconical microcolumn. In fact, it has been recommended that the contact time between solid substrates and extracting reagent for Pb in the earlier steps of sequential extraction protocols (e.g., acid-soluble fractions) should be reduced to the extent possible to minimize the opportunity of readsorption to take place [2,45], which would otherwise lead to underestimation of the content of readily mobilizable Pb [46].

For validation purposes, the repeatability and accuracy of fractionation analyses were for both proposed systems ascertained. Relative standard deviations for extractable and residual concentrations were below 15% in both dynamic methods, thereby confirming that SI-MCE and SI-SFCE are reliable for BCR fractionation of NIST 1633b. Yet, it was not feasible to compare the repeatability of our systems with earlier batchwise BCR tests reported in the literature for NIST 1633b [47] because neither the standard devia-

Table 3 Extractable amounts of trace elements in NIST 1633b coal fly ash as obtained by dynamic BCR fractionation exploiting SI-MCE and SI-SFCE

Element	Concentration, mg kg^{-1}							Recovery %[a]
	Step I	Step II	Step III	Residue	Total (mass balance)[b]	Microwave digestion	Certified value[c]	
Microcolumn extraction								
Cr	6.8±0.1	8.7±0.2	2.6±0.4	156.9±4.4	175.0±4.4	185.1±4.1	198.2±2.4	94.5
Cu	15.7±0.7	3.8±0.2	8.9±0.8	89.2±4.3	117.6±5.1	111.8±2.6	112.8±1.3	105.2
Ni	2.5±0.5	1.4±0.1	3.0±0.3	93.8±0.3	100.7±0.5	104.4±3.5	120.6±0.9	96.4
Pb	4.4±0.1	4.4±0.4	<LOD	52.7±0.7	61.5±1.0	55.1±8.8	68.2±0.6	111.7
Zn	9.8±0.1	2.7±0.8	9.7±1.1	173.6±5.7	195.7±4.4	187.5±6.2	(210)[d]	104.3
Stirred-flow chamber extraction								
Cr	7.0±0.2	7.7±0.2	3.0±0.2	163.4±3.5	181.2±3.5	185.1±4.1	198.2±2.4	97.9
Cu	14.3±0.9	4.0±0.8	7.2±0.4	82.9±1.7	108.4±1.7	111.8±2.6	112.8±1.3	97.0
Ni	2.1±0.2	1.2±0.2	2.7±0.2	99.1±2.2	105.2±2.3	104.4±3.5	120.6±0.9	100.8
Pb	2.9±0.1	3.6±0.5	1.9±0.1	48.2±0.6	56.6±1.0	55.1±8.8	68.2±0.6	103.7
Zn	11.1±1.3	3.5±0.6	8.0±0.3	160.4±2.5	183.0±2.6	187.5±6.2	(210)[d]	97.6

Results are expressed as the mean of three replicates ± standard deviation. LOD_{Pb}, 0.032 mg kg^{-1} in H_2O_2 ($3\sigma_{blank}$)
[a] Calculated as the concentration ratio between mass balance and microwave digestion
[b] Summation of leachable concentrations in steps I, II, and step III plus residual concentration
[c] Certified concentrations given as mean ± standard deviation
[d] Recommended (no certified) value

Table 4 Percentage recoveries of trace elements in NIST 1633b coal fly ash exploiting dynamic SI-MCE and SI-SFCE and comparison with batchwise steady-state extraction

Elements	Step I (%)	Step II (%)	Step III (%)	Extractable metal (%)
Cr[a]	2.0	3.0	3.0	8.0
Cr[b]	3.2	5.2	1.4	9.8
Cr[c]	3.8	4.1	1.6	9.6
Cu[a]	7.0	4.0	4.0	15.0
Cu[b]	14.0	3.4	7.9	25.4
Cu[c]	13.7	3.6	6.4	23.7
Ni[a]	23	21	18	62
Ni[b]	2.4	2.1	2.9	7.3
Ni[c]	2.0	1.2	2.6	5.8
Pb[a]	1.0	5.0	3.0	9.0
Pb[b]	8.0	7.9	0.0	16.0
Pb[c]	5.2	6.5	3.5	15.3
Zn[a]	4.0	3.0	3.0	10.0
Zn[b]	5.2	1.4	5.2	11.8
Zn[c]	6.2	1.9	4.3	12.3

[a] Result reported by Van Herreweghe et al. [47] using batchwise BCR extraction
[b] Results obtained in this work by SI-MCE
[c] Results obtained in this work by SI-SFCE

tions nor the coefficients of variation of results were mentioned.

Method accuracy for SI-MCE and SI-SFCE was evaluated by summation of extractable concentrations in the acid-soluble, reducible, and oxidizable fractions plus residue for each individual trace element and comparison of the mass balance with the certified/recommended values or total concentrations estimated by microwave digestion (MWD). As shown in Table 3, recoveries for Cu, Ni, Pb, Zn, and Cr ranged from 94.5% to 111.7% and 97.0% to 103.7% for SI-MCE and SI-SFCE, respectively. In fact, the *t* tests of comparison of means between cumulative amounts and MWD concentrations [44] confirmed the inexistence of significant differences at the 0.05 significance level for the overall fractionated trace elements in both SI extraction systems. Hence, both SI-MCE and SI-SFCE methods are free from both additive and multiplicative matrix interferences, making the application of the method of the standard additions unnecessary.

The extractable amounts of trace elements in NIST 1633b as obtained by flow-through dynamic BCR leaching in SI-MCE and SI-SFCE were critically compared with batchwise BCR sequential extraction results reported by Van Herreweghe et al. [47] for NIST 1633b who utilized exactly the same reagents as for the SI approaches (see Table 4). The analysis time per fraction in dynamic fractionation amounted to 75 min for both the acid-soluble and reducible fractions and 50 min for the oxidizable fraction, thus giving rise to a total analysis time of 3.3 h in contrast to 48 h needed in classical BCR. As a consequence of the exhaustive extraction nature of flow-through fractionation tests, reagent expenses in SI-MCE and SI-SFCE are larger than those involved in batchwise methods. Thus, cumulative volumes of 150 mL of acetic acid and hydroxylammonium and 100 mL of hydrogen peroxide were employed in both automated BCR extraction assemblies as compared to 40 mL of acetic acid and hydroxylammonium and 20 mL hydrogen peroxide in batchwise BCR for 1 g of solid.

Despite the different operational conditions in steady-state and dynamic extraction methods and notable differences in overall extraction times, a good agreement was found for cumulative extractable amounts of Cr and Zn, as confirmed by application of a *t*-test of comparison of means [44]. For Cu and Pb, however, both SI-fractionation methods gave rise to improved percentage extractability. This is most likely due to the fact that the release of a given target element in batchwise methods is largely influenced by the solubility products of salts. Most importantly, the extractions of highly alkaline fly ash samples might not be performed in steady-state methods under the nominal extractant pH [9]. On the contrary, dynamic flow-through methods are capitalized on the continuous renewal of the leaching phase, with the consequent displacement of solubility equilibria and performance of leaching tests under nominal pH values, thereby fostering a more accurate quantitation of mobile pools for worse-case scenarios.

The most surprising information encountered when examining Van Herreweghe's article [47] is the tenfold improved extractability of Ni as compared to the proposed dynamic flow methods. Nevertheless, the SI-MCE and SI-SFCE results for Ni are in good agreement with other

articles reporting batchwise equilibrium-based fractionation of fly ashes, where extractable percentages of Ni exploiting the three-step BCR leaching test ranged from 4% to 11% [31, 48, 49]. Therefore, we attributed Van Herreweghe's data to potential typographical error in the presentation of experimental results.

Conclusion

In this paper, the analytical performances of two novel automated fractionation schemes, the so-called SI-MCE and SI-SFCE, have been thoroughly investigated for accurate determination of overall mobilizable pools of trace elements in NIST 1633b fly ash exploiting the standard BCR leaching test. The most relevant assets of the proposed flow-through methods, as demonstrated by application of experimental designs, is that within the investigated intervals, neither the solid to liquid ratio nor the extraction time is a critical variable for total metal leachability as a consequence of the multistage extraction nature of dynamic fractionation. The significant decrease of analysis times in both SI-based BCR fractionation methods, namely, 3.3 h as compared with 48 h for batchwise BCR is also worth mentioning

We conclude that both SI-MCE and SI-SFCE, as opposed to the steady-state BCR test, are not operationally defined procedures, whereby the extraction conditions, e.g., sample amount and solid-to-liquid ratio, could be readily modified attending to the needs of the particular assay (e.g., sample availability and representativeness) without statistically significant changes on BCR metal percentage leachability, provided that exhaustive extraction is ensured and appropriate sample containers are designed.

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CHAPTER 5

Automated dynamic chemical fractionation method with detection by plasma spectrometry for advanced characterization of solid biofuels

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PAPER

Automatic dynamic chemical fractionation method with detection by plasma spectrometry for advanced characterization of solid biofuels†

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A flow-through dynamic leaching test capitalizing on sequential injection (SI) stirred-flow chamber (SFC) extraction is proposed as a front end to inductively coupled plasma optical emission spectrometry (ICP-OES) for automatic and expedient evaluation of potentially mobilisable pools of ash-forming elements (*viz.*, K, Na, Ca, Mg) in troublesome solid biofuels (namely, pine twigs) and ascertainment of the kinetics of metal release. A three-step sequential extraction scheme exploiting increasingly aggressive solvents and encompassing distilled water, 1.0 mol L^{-1} ammonium acetate and 1.0 mol L^{-1} hydrochloric acid is herein selected for partitioning of targeted elements in the water-soluble, the exchangeable and the acid-soluble nominal fractions, respectively. The relative amounts of elements in the first two steps are regarded as valuable measures of the release of reactive inorganic species into the flue gases in the course of combustion in boilers and furnaces with the subsequent associated fireside problems. The SI-SFC-based setup coupled to ICP-OES features high tolerance to flow rates (≤2.5 mL min^{-1}) without pressure drop and affords more accurate evaluation of accessible pools of ash-forming matter compared to equilibrium-based methods as a consequence of the exhaustive extraction nature of dynamic fractionation, the solid to liquid equilibria shift, the absence of metal redistribution effects and the release of metals under the nominal pH of the extractant. No significant differences were found at the 0.05 significance level between summation of leached concentrations in each step plus residual fraction and total concentration of ash-forming elements in twigs as determined by microwave digestion, thus revealing the reliability and lack of bias (trueness) of the automatic method. The overall extractable pools of ash-forming elements were quantified in merely 3 h *versus* 7 days in batchwise leaching tests. The automatic hyphenated technique is thus suitable for expeditious advanced characterization of woody biofuels so as to assist in deciding whether firing biomass fuels leads to potential corrosion risks in combustion devices.

Introduction

Biomass is a contemporaneous (non-fossil) and complex biogenic organic–inorganic solid product generated by natural and anthropogenic (technogenic) processes, and comprises natural constituents originating from growing land- and water-based vegetation and related technogenic processed products thereof.[1] Biomass fuels or biofuels play a major role in reducing the reliance on fossil fuels (namely, coal, peat, oil and natural gas) by making use of thermo-chemical conversion technologies.[1–3] In fact, an increasing global awareness about environmental issues, in the current efforts to decrease the net CO_2 emissions to the atmosphere, is acting as the driving force behind the use of alternative and renewable organic sources of energy (namely, fast growing grasses, trees, and agricultural residues including used vegetable oil, wheat straw, or corn stalks among others)[2–4] to promote bioenergy as a channel to counter environmental issues.[5]

Woody biomass fuels can be categorized into three groups based on their origin in trees: wood fuels, bark fuels, and logging (forest) residue fuels.[6] The elemental and mineralogical compositions and physical properties are a function of the biomass fuel type and origin, of the harvesting season of biomass crops, and of collection practices, storage and transportation.[1,6–8] Biomass processing systems constitute a significant portion of the capital investment and operating costs of a woody biomass conversion facility. Investment is not merely allied to the biomass source but to the feedstock preparation requirements and the alkali contents in the fuels to be processed as well. The chemistry of inorganic transformations of solid biofuels in the boilers is quite complex, involving multiple

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physicochemical pathways among alkali, alkaline earth, and other inorganic and organic constituents in the fuel. Inorganic components involved in ash formation from biomass resources include primarily alkali and alkaline earth elements (*viz.*, K, Na, Ca, Mg).[1,5,6,8–11] These inorganic elements influence the combustion process by forming gaseous emissions and solid deposits, changing the ash melting behavior and most importantly, accounting for ash-related fireside pitfalls encompassing slagging in furnaces, fouling of heat exchangers, and bed agglomeration and sintering in fluidized bed combustion devices.[6,12–17] Therefore, it is of utmost relevance to gain in-depth insight into the amounts of ash-forming elements in biomass fuels along with the chemical reactions and potential fouling and corrosion effects they might cause during combustion so as to optimize the design of the combustion device and setting quality control tools for the biomass fuels to be fired. Consequently, detailed physicochemical characterization of biofuel resources plays a critical role in the development of new biomass furnaces, boilers and process control systems to accomplish more efficient combustion technologies and propose pertinent measures whenever needed for fuel pretreatment prior to firing.

Standard fuel analysis, including the proximate analysis for prediction of burning characteristics, the ultimate analysis for estimation of flue gas composition and the heat value, are the first and most important properties to be established for any new, unknown biomass fuels or biomass fuel blends. However, experience has shown that these traditional analysis results are of scarce significance when investigating fuel ash behavior, even though they would embrace standard ashing and ash melting tests.[6,13,18]

To tackle this problem, chemical fractionation methods have been evolved as advanced fuel characterization tests to assist in understanding how ash-forming elements are tied up in the fuel by discrimination of chemical forms of elements according to the step-wise release in a suite of increasingly aggressive extraction solvents.[4–6,11,16,18–22] In most cases, a three-step sequential extraction scheme is exploited for investigation of leaching patterns in a sequence of distilled water, 1.0 mol L^{-1} ammonium acetate and 1.0 mol L^{-1} hydrochloric acid.[4–6,11,16,19–21] The first step in biomass fractionation is intended to soak the fuel so as to dissolve the water soluble components including alkali and alkaline earth metal salts, mostly potassium, sodium and calcium chlorides. In the second step, ion exchangeable elements, such as organically associated sodium, calcium and magnesium (nominal phases), are replaced with ammonium ions and thus released into the liquid phase. The relative amounts of elements in the first two steps are regarded as valuable measures of the release of reactive inorganic species into the flue gases in the course of combustion because the vaporization and subsequent chemical reactions of the associated salts are responsible for much of the fouling, sulfation, corrosion, bed sintering and slagging in combustion devices and fluidized-bed reactors.[5,11,13,16,18–20] The increase in leachable forms of elements in these steps is thus related to enhanced risks of ash deposition on the boiler and the head exchanging surfaces, which in turn would lower process performance. The third extraction step is aimed to leach phases including acid soluble compounds. The residue remaining after leaching usually contains silicates and elements that are covalently bound to organic matter. This residual fraction of immobilized alkali along with that of the acid-leachable step are considered less reactive in combustion.[5,11,13,16,19,20]

Even though the equilibrium-based three-step chemical fractionation method is a well accepted tool for alkali and alkaline earth metal partitioning in woody biomass, its actual applicability is at present hampered by the length of the overall procedure and the large number of manual operations involved.[4,6,11,16,19–21] In fact, it may take months before leaching results become available to intended parties making the method useless for fast screening of potential risks in firing challenging fuels (*e.g.*, twigs) and fuel blends, and, hence, less suited for routine analyses. In addition, the significance of results from batchwise fractionation is still debatable because of potential phase overlapping and re-adsorption/re-distribution of leached elements in the course of mild extractions.[23–25]

To overcome the above shortcomings, a novel approach for automation of leaching tests of pine twigs (taken as a model sample) is herein proposed for accurate quantitation under worst-case scenarios of the soluble metals involved in corrosion of combustion devices (namely, K, Na, Ca, Mg) on a short notice. The analytical setup capitalizing on in-line hyphenation of Sequential Injection (SI)-based stirred-flow chamber (SFC) extraction to inductively coupled plasma-optical emission spectrometry (ICP-OES) is devised to overcome pitfalls of former dynamic sequential extraction procedures for risk assessment of environmental solid substrates. The main pitfalls include the limited sample amount to be handled (mostly $\leq$30 mg),[26–32] the undue backpressure in flow systems furnished with peristaltic pumps,[26,29,33–35] the restrictions in soil particle dimensions (*e.g.*, $\leq$1.5 mm)[36,37] and the manual exchange of extractants,[26,29,34,36,38,39] making them ill-suited for automatic fractionation of heterogeneous biomass fuels. To the best of our knowledge, no automatic dynamic chemical fractionation method affording kinetic data has been reported so far for simplification and acceleration of advanced biofuel characterization.

Experimental

Reagents and solutions

All reagents were of analytical grade and solutions thereof were prepared using Milli-Q water (Purelab®Ultra, ELGA LabWater, Woodridge, IL). A 1.0 mol L^{-1} ammonium acetate solution was prepared by dissolving 38.5 g of ammonium acetate (Merck, Darmstadt, Germany) in 1 litre of Milli-Q water. A 1.0 mol L^{-1} hydrochloric acid solution was prepared by diluting of 83.2 mL of concentrated hydrochloric acid (37%, J.T. Baker, Deventer, The Netherlands) up to 1 litre. Nitric acid (65%, J.T. Baker) and hydrogen peroxide (30%, Merck) were used as oxidizing reagents for microwave digestion of biomass fuels and residues left over after fractionation.

Sampling and sample preparation

Twigs were selected as troublesome model samples for in-line dynamic chemical fractionation because of significant swelling in aqueous media and floating on the leaching solutions in the stirred-flow chamber.

A *ca.* 100-year old Scots pine (*Pinus sylvestris* L.) was harvested from a small stand in the municipality of Kronoby (Midwest Finland). The sampling site was located *ca.* 20 km from the coast of the Gulf of Bothnia and 1 km away from any road. The thinnest branches (<1.0 cm), the so-called twigs, containing both wood and bark, were collected as a whole together with the foliage. In the laboratory, the foliage was separated and the twigs were oven-dried at 105 °C until constant weight. Prior to analysis, the samples were grinded to a particle size less than 1 mm in a bench mill[8] and placed in sealed polyethylene bags in dark storage. Particular attention was paid to ensure a representative test portion for ensuing chemical fractionation assays.

Hyphenated flow set up for automatic chemical fractionation and in-line analyses of extracts

An FIAlab-3000 (FIAlab, Bellevue, WA) sequential injection system equipped with an internally incorporated 6-port multiposition selection valve (SV) and a 30 000-step syringe pump (SP) (Cavro, Sunnyvale, USA) with a capacity of 5 mL was used for automatic handling of leaching reagents and delivery of well-defined volumes of extractants to the solid biofuel as contained in a SFC. Milli-Q water was used as a carrier solution. The central port (CC) of the SV was connected to the SP *via* a holding coil (HC), which consisted of a 3.0 m long polytetrafluoroethylene (PTFE) tubing (1.5 mm i.d.) with an internal volume of *ca.* 5.3 mL. The outlets of the SV were connected to the extractant reservoirs, SFC or waste through rigid PTFE tubing (1.5 mm i.d.) using polyetheretherketone (PEEK) fittings. The SFC was connected to the SI setup as an external module. A sketch of the experimental setup for advanced dynamic chemical fractionation of twigs is schematically shown in Fig. 1. The automatic analytical sequence (see below) comprises the consecutive aspiration of the individual extractants from different external ports of the SV which are sequentially exposed to the sample. The SI system is fully computer controlled *via* the user-friendly FIAlab associated software for Windows 5.0.

The SFC was designed to contain a weighed amount of twigs and to allow extractants to flow sequentially so as to successively leach metals from targeted phases. The SFC and cover thereof were constructed from borosilicate glass with a nominal volume of *ca.* 15 mL, as described elsewhere.[33] A rubber gasket was placed on top of the SFC followed by a nylon filter (GE Osmonics Labstore, MN) of 0.45 μm pore size and 47 mm diameter to allow the dissolved matter to flow through but retain solid particles. The SFC is completed with a second rubber gasket and the cover on top of the extraction chamber. The inlet of the SFC is connected to the SV of the SI manifold (central unit). The outlet is allied to a secondary flow system for in-line dilution of extracts prior to ICP-OES analysis with concomitant

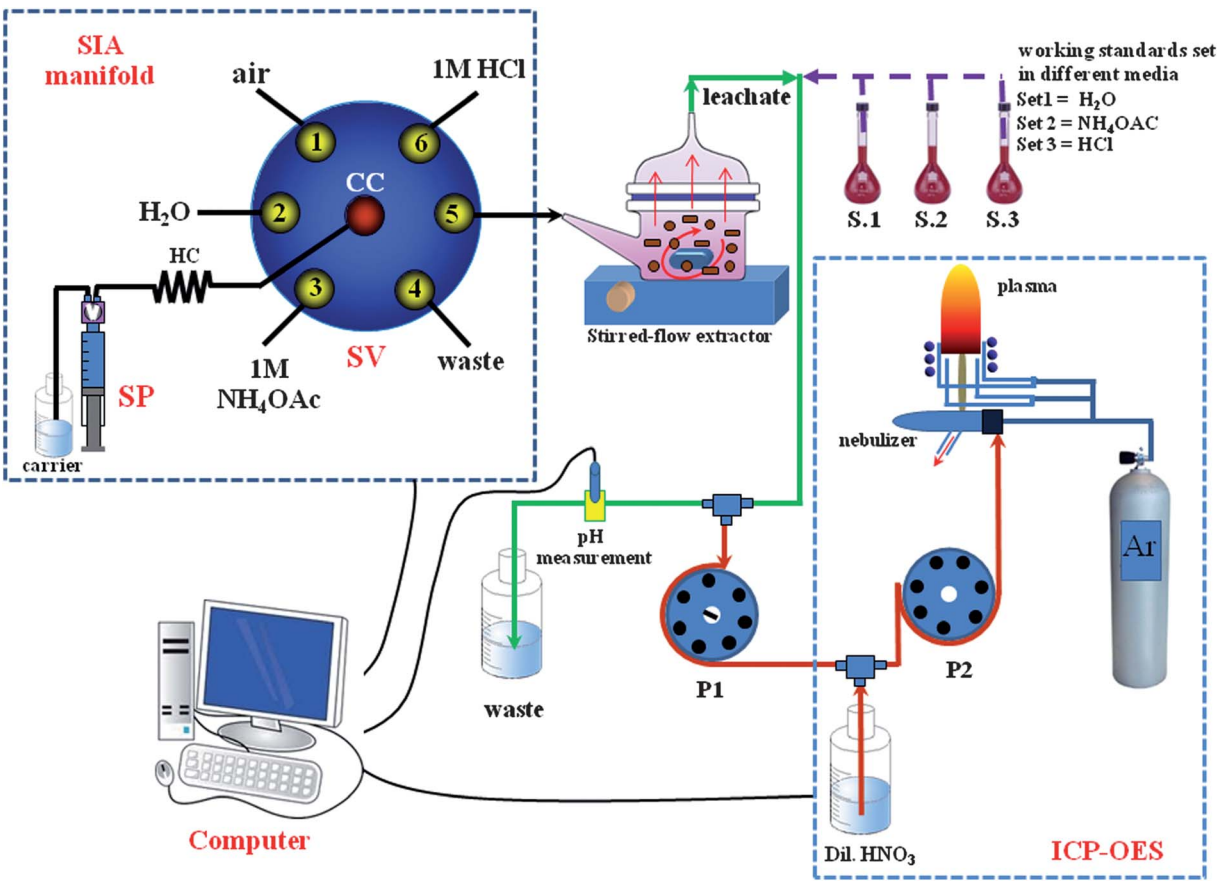

Fig. 1 In-line coupling of stirred-flow extraction chamber to ICP-OES for automatic chemical fractionation of ash-forming elements in twigs. SP: syringe pump; SV: multiposition selection valve; P: peristaltic pump; CC: central communication channel. The figure also illustrates the matrix-matched external calibration procedure.

real-time pH measurement using a PASPORT pH sensor (PS-2102, Pasco, Roseville, CA, USA) in combination with Data Studio software. For the in-line dilution of the extracts, an ancillary peristaltic pump (P1) (Gilson, Minipuls 2, Villiers le Bel, France) was utilized for sampling the main leachate stream at a given flow rate through a T-connector (see Fig. 1). This secondary stream merges downstream with a steady flow of 2% (v/v) HNO_3 by programming the rotation speed of peristaltic pump P2, which feeds the ICP-OES nebulizer. The dilution factor is thus determined by the P2 to P1 flow rate ratio.

Analytical protocol

A weighed dry sample (usually 150 mg twigs) was transferred to a clean SFC together with a small magnetic bar (1 cm long) and the overall components of the SFC container were tightly clamped. The SFC was placed on a magnetic stirrer (Agimatic-E, P-Selecta, Spain) which was set to 500 rpm. For real-time monitoring of the soluble metal species, the SP was programmed to aspirate 100 μL of air (port 1 of the SV to prevent extractant dispersion in the HC) and 4900 μL of distilled water in 14 s (port 2) consecutively into the HC. Thereafter, the flow was reversed and the extractant plug perfused the biomass fuel containing SFC at 2.5 mL min^{-1} while retaining the air segment within the HC. The main leachate stream was propelled to the 3 mL flow-through cell of the pH sensor for in-line pH detection preceded by continuous sampling of 0.17 mL leachate per minute into the secondary stream. In this work, P2 was programmed to continuously aspirate the composite leachate/HNO_3 solution at 1.5 mL min^{-1}, as a result of which the main leachate stream was 9-fold diluted. The above-mentioned automatic procedure was repeated twenty-four-fold for the water-soluble fraction until the ICP-OES readouts reached the baseline, thus indicating exhaustive extraction of the targeted phase. The SP was refilled at a high flow rate, that is, 21 mL min^{-1}, to prevent drawing excessive solution from the tube connected to waste into ICP-OES. Thereafter, the SV was switched to port 3 for aspiration of the first plug of 4900 μL of 1.0 mol L^{-1} ammonium acetate for extraction of ion-exchangeable metals. A virtually identical procedure as described above for water extraction was undertaken with a total extractant volume of 118 mL regardless of targeted metal species. Prior to changing to the last fraction (1.0 mol L^{-1} HCl at 70 °C), the content of the SFC was thoroughly rinsed with 29.4 mL of Milli-Q water for fast leaching of the acid-soluble species at the nominal pH of the extractant. As per Benson's fractionation method,[40] the entire flow manifold components including HC, SFC and HCl reservoir were placed in a thermostatic bath to assure this extraction step to occur at 70 ± 5 °C. Experimental results revealed that the acid soluble calcium was released in ≤120 mL of 1.0 mol L^{-1} HCl at 70 °C. Afterward, a second washing step with 29.4 mL of Milli-Q water was implemented before collection of the twig residue for determination of the pool of immobile metal species. A detailed operational sequence of the SI procedure is listed in Table S1 (ESI†).

A five point matrix matched external standard calibration was exploited for determination of target elements in the twig extracts. To this end, the SFC was replaced by a set of multi-element standard solutions (Merck) introduced into the ICP-OES in a continuous-flow mode as illustrated in Fig. 1.

Analytical instrumentation

The leachate stream of the SI-SFC setup was analyzed at real-time exploiting a Perkin-Elmer Optima 5300 DV inductively coupled plasma optical emission spectrometer (ICP-OES, Perkin-Elmer, MA, USA) equipped with a GemTip Cross-flow pneumatic nebulizer (Perkin-Elmer). The operating conditions of the ICP-OES are given as follows: RF power, 1300 W; plasma Ar flow rate, 15 L min^{-1}; auxiliary Ar flow rate, 0.2 L min^{-1}; nebulizer flow rate, 1.5 mL min^{-1}; read time, 5 s; view mode, axial and analytical wavelengths, 227.546 and 317.922 nm for Ca, 404.721 and 766.497 nm for K, 279.077 and 285.213 nm for Mg, and 330.237 and 589.592 nm for Na. The instrument readouts were recorded every minute for construction of the leaching profiles. The amount of leachable elements in twigs was evaluated as the product of the area of the extraction profiles (concentration *versus* time) and the extractant flow rate.

Total digestion of twigs and residues

The residue left over after dynamic chemical fractionation was collected and digested in a closed-vessel microwave digestion system (MLS-1200 Mega) from Milestone (Sorisole, Italy) for mass balance validation. Solid biomass residues were carefully transferred to PTFE vessels to which 5 mL of HNO_3 and 3 mL of H_2O_2 were added. The first microwave digestion program composed of seven consecutive steps is specified in Table 1 and followed the recommendations by Stals *et al.*[41] After cooling down to room temperature, samples were subjected to a second digestion protocol with further addition of 2 mL of HNO_3 and 1 mL of H_2O_2 as detailed in Table 1. An identical procedure was utilized for determination of the total concentration of elements in 0.15 g of raw twigs. Digests were finally diluted to 50 mL with Milli-Q water.

Results and discussion

The idea behind this work is to design an expedient alternative to classical methods for investigation of the reactive inorganic

Table 1 Microwave digestion procedure for total dissolution of twigs

Time/min	Power/W
Step 1: added to the samples: 5 mL HNO_3 + 3 mL H_2O_2	
2	200
1	0
5	200
5	340
5	400
20	340
10	Ventilation
Step 2: added to the samples after cooling down: 2 mL HNO_3 + 1 mL H_2O_2	
5	200
5	340
5	400
5	500
15	340
10	Ventilation

content in biomass fuels so as to predict potential boiler failure. It is worth noting that the proposed arrangement fosters in-line dilution of extracts at will by tailoring the ratio of the flow rate of the secondary stream (uptaken by P1) and that of the ICP sample introduction system (see Fig. 1), and thus admits extracts with varied concentration levels of alkalis and alkaline-earth elements.

Earlier works in the literature coping with the coupling of stirred-flow chamber extraction with atomic emission spectrometry exploiting flow-injection networks reported the lack of a steady leachant flow rate throughout the fractionation protocol.[33,34] In our work, the replacement of the peristaltic pump with a high-precision microsyringe pump resulted in a flow setup capable of enduring the flow impedance caused by fine particle clogging of the membrane filter of the reactor when using extraction flow rates up to 2.5 mL min^{-1}.

Leachate pH profiles

The leaching behavior of elements in biomass fuels should be interpreted in terms of solubility equilibria and leachate pH, which determines the correlation between nominal phases and those actually released under given experimental conditions.

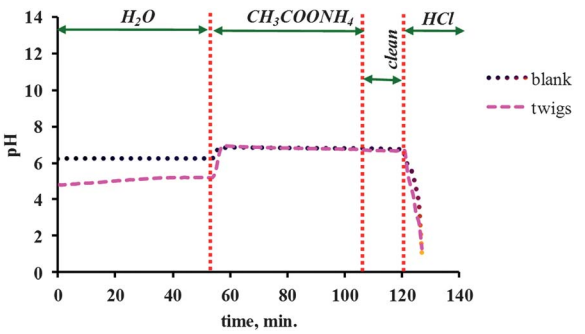

Fig. 2 Leachate pH profiles of twigs as obtained by in-line recording of the extract pH in the course of dynamic chemical partitioning of ash-forming elements.

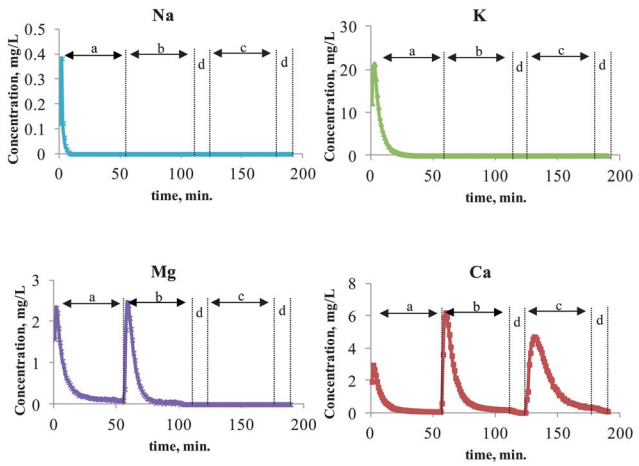

Fig. 3 Average extractograms of ash-forming elements in twigs exploiting three-step SI-SFC fractionation with in-line detection of extracts by ICP-OES ($n = 3$): (a) water soluble fraction, (b) exchangeable fraction, (c) acid soluble fraction, (d) water cleaning fraction.

Table 2 Extractable amounts and mass balance validation of ash-forming elements in twigs using in-line dynamic and batchwise equilibrium extraction

	H_2O		CH_3COONH_4		HCl		Residue		Total leachable metal[b]		Total[c] Microwave digestion/ mg kg^{-1}	Recovery[d]	
Element	In-line/ mg kg^{-1}	Batchwise/ mg kg^{-1}	In-line/ mg kg^{-1}	Batchwise/ mg kg^{-1}	In-line/ mg kg^{-1}	Batchwise/ mg kg^{-1}	In-line/ mg kg^{-1}	Batchwise/ mg kg^{-1}	In-line/ mg kg^{-1}	Batchwise/ mg kg^{-1}		In-line (%)	Batchwise (%)
Twigs													
Na	11.3 ± 0.3	19 ± 2	nd[a]	nd[a]	nd[a]	nd[a]	153 ± 3	143 ± 13	11.3 ± 0.3	19 ± 2	167 ± 5	98	97
K	2344 ± 32	1975 ± 113	nd[a]	nd[a]	nd[a]	nd[a]	321 ± 2	365 ± 12	2344 ± 32	1975 ± 113	2667 ± 35	100	88
Mg	358 ± 18	320 ± 26	337 ± 7	234 ± 26	nd[a]	nd[a]	25.4 ± 0.9	54 ± 8	695 ± 25	554 ± 52	716 ± 9	101	85
Ca	409 ± 16	223 ± 11	1153 ± 36	586 ± 52	1507 ± 20	1763 ± 47	1455 ± 30	1725 ± 73	3069 ± 61	2572 ± 37	4636 ± 24	97	93

[a] nd: not detected. [b] Summation of leachable concentrations in water, ammonium acetate and hydrochloric acid steps. [c] Total metal content in the original sample obtained from microwave digestion. [d] Calculated as the ratio between mass balance (total leachable metal plus residue) and total metal content.

Unfortunately, the actual leachate pH in batchwise fractionation procedures for biomass fuels is seldom reported in the literature, and mere indications of nominal pH values for ammonium acetate and hydrochloric acid are given.[21] In fact, our own results of steady-state chemical fractionation revealed that the pH of ammonium acetate leachates of twigs merely reached a range of values of 5.9–6.0 (<7.0) after 24 h of stirring. Thus, the pools of mobilizable elements are most likely underestimated. On the contrary, the dynamic leaching test used in this work involves a multistage, exhaustive extraction process, whereby the leachate pH gradually changes until reaching the nominal extractant pH (excepting for unbuffered water), regardless of the buffer capacity of the raw biomass. The proposed SI-SFC fractionation method should thus be regarded as an appealing tool for better estimation of the potentially available fractions of alkali and alkaline-earth elements under worst case scenarios.

The leachate pH patterns (including the nominal extractant pH) in the course of the dynamic fractionation test are illustrated in Fig. 2. For the water-soluble fraction of twigs, the initial acid pH (pH < 5) is a consequence of the release of organic acids,[6] and the dissolution of potassium dihydrogen phosphate salts as well. Upon changing the extractant composition to ammonium acetate, the pH is rapidly sustained in less than 6 min whereupon extraction takes place at the nominal pH of the extractant. The pH electrode was disconnected from the system at pH < 1 because of inaccurate measurements in a strong acidic extraction milieu.

Exploration of metal release in biomass fuels

The transient leaching pattern obtained by a graphical plot of the concentrations of leached elements against extraction time is called an extractogram.[34,42,43] Fig. 3 depicts the extractograms of Na, K, Mg and Ca in twigs obtained in-line by coupling of SI-SFC with ICP-OES. The fresh extractant is continuously delivered to the SFC until the metal in the target phase is completely leached as seen from the signals in Fig. 3 gradually leveling off to the baseline level. Peak widths of ash-forming elements indicate how loosely or strongly bound to the parent biomass phase is the targeted element. Further, the amount of elements mobilizable in each individual fraction is readily determined *via* peak area measurements in the extractograms whenever the *X*-axis represents the extractant time.

The most critical ash-forming element from a fireside-problem standpoint is potassium. This element is the root cause, during thermo-chemical biomass conversion, of bed sintering, fouling and corrosion on the boiler's heat transfer surface. Streamlined quantification of the reactive pool of potassium is to be realized in our system by automatic analysis of the water-soluble and exchangeable extracts. The insoluble part of potassium is oftentimes encountered as silicate (clay) contaminations of the fuel, the magnitude of this pool being determined by resorting to hydrochloric acid as the extractant. This third fraction is deemed to be inert and is not involved in the lively chemistry of the more reactive chemical forms of potassium in the flue gases. The extractograms in Fig. 3 and results in Table 2 demonstrated that over 88% of potassium in twigs is found in the most reactive phase, most likely associated to chloride salts. Most importantly, the in-line extractograms revealed that the water soluble potassium is leached in less than 25 min *in lieu* of 24 hours as endorsed in batchwise fractionation methods. This also accrues with sodium which shares a similar chemistry with potassium in combustion devices and accounts for sulfate deposits on boiler surfaces, yet the water-soluble concentration of sodium in twigs was proven to be 30 times lower.

As opposed to alkalis and magnesium (see Fig. 3 and Table 2), calcium is mostly leached in the ammonium acetate and acid fractions, accounting for 38% and 47%, respectively, of total available calcium. This is a consequence of the fact that calcium is a prevailing constituent of cell walls and other organic components of cell structures, mostly carboxylic acids, thus bearing a largely ion-exchangeable character. Organically associated calcium and magnesium compounds as determined in the second step of the leaching test will be converted into finely

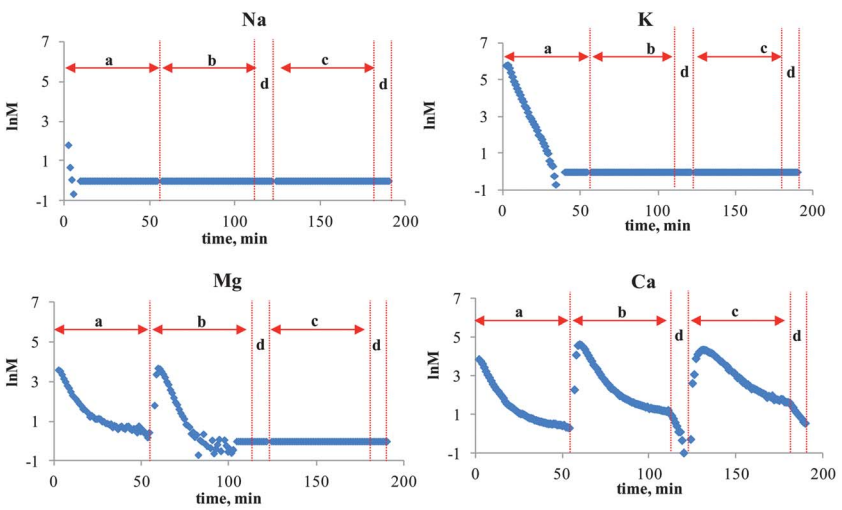

Fig. 4 Kinetic curves of ash-forming elements in twigs as obtained by plotting the natural log of removal rates (ln M_t) against time. Kinetic parameters are calculated from the linear segments by resorting to a first-order reaction model. (a) Water soluble fraction, (b) exchangeable fraction, (c) acid soluble fraction, (d) water rinsing fraction. See text for further details.

divided lime (CaO) and magnesia (MgO) particles[44] under hot furnace conditions, and thus regarded as quite reactive forms in the furnace and flue gases.[13]

The dynamics of leaching processes of ash-forming matter in biomass resources are not fully understood to date. Thus, significant efforts were devoted in this work to the investigation of the liability of metal associations in twigs *via* modeling of the plots of cumulative concentrations *versus* time using the so-called two-first-order reaction kinetic model.[45,46] The average extracted metal rate per time unit (M_t) may be expressed as follows:

$$M_{t_{i-1}<t<t_i} = \frac{(C_{t_i} - C_{t_{i-1}})}{t_i - t_{i-1}} \quad (1)$$

where C_{t_i} (mg kg^{-1}) represents the cumulative concentration of a given alkali or alkaline earth element extracted in each step of the fractionation test at time t_i. The two first-order model assumes that M_t can be modeled by the sum of two distinct pools of ash-forming elements occurring simultaneously, each being described by an exponential decreasing function[45] as detailed below:

$$M_t = A_0(e^{-\alpha t}) + B_0(e^{-\beta t}) \quad (2)$$

where A_0 (mg kg^{-1} min^{-1}) stands for the initial removal rate of the readily extractable (labile) metal fraction and B_0 (mg kg^{-1} min^{-1}) for that of the slowly extractable (moderately labile) metal fraction associated with the apparent rate constants α (min^{-1}) and β (min^{-1}), respectively.

Notwithstanding the fact that this model has been merely applied in the literature for investigation of single extractions involving strong, non-selective leaching reagents, such as EDTA or citrate,[45–47] the plot of ln M_t *versus* time for twigs in each step revealed one or two linear segments (see kinetic curves in Fig. 4) for the suite of target elements. The coefficients of correlation are ≥0.98 (see Table 3), indicating that almost the overall variability has been accounted for with the variables specified in the model (see eqn (2)). This finding demonstrates the usefulness of the model for the operationally defined speciation of ash-forming elements in terms of leaching and the quantification of kinetic parameters in the release of target elements from twigs (see Table 3). The initial metal removal rates are dominated by the parameter A_0 characteristic of the pool of labile elements, yet its contribution decreased rapidly to become negligible whereby M_t can be estimated on the basis of the second term (slowly leachable elements) at greater extraction times. The initial extraction rates and kinetic parameters for fast and slowly leachable fractions were calculated from the *Y*-axis intercepts and the slopes of the linear fittings, respectively (see Table 3). The kinetic model confirmed that the overall available K in twigs is readily extractable ($\beta \approx 0$) with an elevated initial extraction rate and thus severe ash slagging risks in combustion devices might be anticipated whenever twigs are used as a pristine or blended biofuel.

Table 3 Estimation of initial extraction rates (A_0 and B_0) and apparent leaching rate constants (α and β) of ash-forming elements in the 3-step dynamic fractionation method for the two pools specified in the first-order mathematic model for twigs

Metal	Water fraction						Ammonium acetate fraction						Hydrochloric acid fraction					
	A_0/ mg kg^{-1} min^{-1}	α/ min^{-1}	R^2	B_0/ mg kg^{-1} min^{-1}	β/ min^{-1}	R^2	A_0/ mg kg^{-1} min^{-1}	α/ min^{-1}	R^2	B_0/ mg kg^{-1} min^{-1}	β/ min^{-1}	R^2	A_0/ mg kg^{-1} min^{-1}	α/ min^{-1}	R^2	B_0/ mg kg^{-1} min^{-1}	β/ min^{-1}	R^2
K	557	0.19	0.9971	N[a]	N[a]	—	N[a]	N[a]	—	N[a]	N[a]	—	N[a]	N[a]	—	N[a]	N[a]	—
Na	27	0.80	0.9819	N[a]	N[a]	—	N[a]	N[a]	—	N[a]	N[a]	—	N[a]	N[a]	—	N[a]	N[a]	—
Ca	63	0.15	0.9925	8.4	0.04	0.9801	199	0.14	0.9969	14	0.03	0.9641	179	0.08	0.9927	107	0.06	0.9768
Mg	51	0.15	0.9913	21	0.08	0.9606	29	0.19	0.9937	LOF[b]	LOF[b]	—	N[a]	N[a]	—	N[a]	N[a]	—

[a] N: null slope and intercept, [b] LOF: lack of fit of the kinetic model.

Method validation and comparison with batchwise fractionation

Estimation of the trueness of the analytical results as obtained with the fully mechanized dynamic leaching test was undertaken by resorting to mass balance validation as a quality control procedure. It is worth mentioning at this point that only the

leachate of each step without replicates is often analyzed in batchwise procedures[21] because of time constraints and cost considerations, whereby reported results are in fact debatable. A survey in the literature revealed that systematic errors are frequently encountered in conventional batchwise tests for woody biomass fractionation as revealed by unsatisfactory material balances.[4,19,21] Mass balance was performed in this work by summation of extractable concentrations in the water, the ammonium acetate and the acid-soluble fractions plus the residue for the suite of target elements and compared with the total amount of elements in the biofuel as estimated by microwave digestion (see Experimental). As shown in Table 2, recoveries of Na, K, Mg and Ca in twigs ranged from 97 to 101%. In fact, the *t*-tests of comparison of means[48] between cumulative amounts (mass balance) and total metal content confirmed the inexistence of significant differences at the 0.05 significance level for the overall fractionated elements in the dynamic SI-SFC extraction system. Hence, the automatic method is free from both additive and multiplicative matrix interferences. The extractable amounts of alkali and alkaline-earth elements in twigs obtained by SI-based dynamic leaching were paralleled with the batchwise counterpart described by Werkelin *et al.*[21] using a solid to liquid ratio of 1 : 10 for water extraction and 1 : 5 for the remainder of the leaching test. Relative recoveries of several elements in the batchwise leaching test (see Table 2) were however down to 85%, because of the large number of manipulations involved, as compared to ≥97% in the automatic and completely enclosed flow setup that ensures the reliability and accuracy of fractionation data. Further, improved extractability of K, Mg and Ca in the water soluble and exchangeable fractions was observed in dynamic fractionation (see Table 2). This is a consequence of the minimization of metal re-adsorption and the multi-stage nature of the procedure. Thus, underestimation of the most reactive forms of elements might be expected in the conventional steady-state methods. Most importantly, the overall extractable pools of ash-forming elements in twigs were determined by the SI-SFC-based dynamic procedure in merely 3 h compared to more than 7 days whenever the batchwise leaching test is used.

Conclusion

The hyphenated setup devised in this work for dynamic fractionation affords fingerprinting of chemical associations of ash-forming elements in woody biofuels on a short notice. Recorded leaching patterns (extractograms) have been herein exploited for streamlined discrimination of reactive against inert chemical forms of ash-forming matter in twigs. The automatic method offers excellent intermediate precision, improved laboratory safety as compared to batchwise counterparts, reduced risks of sample contamination and minimal operational maintenance. Besides, the overall sequential extraction scheme is accomplished in less than 3 hours *in lieu* of 7 days whenever undertaken in a manual fashion. Current efforts are directed to broaden the applicability scope of the in-line coupling of SI-SFC to ICP-OES for fast screening of readily mobilisable forms of alkali and alkaline-earth elements in biomass resources other than twigs, including waste derived fuels (*e.g.*, sewage sludge and agriculture wastes, such as straw), and troublesome fuel blends as well.

Further research is to be focused on the elucidation of associations of ash-forming metals in biomass fuels and biomass ashes as well by resorting to energy dispersive X-ray fluorescence spectrometry and X-ray diffractometry.

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CHAPTER 6

Elucidation of associations of ash-forming matter in woody biomass residues

Elucidation of associations of ash-forming matter in woody biomass residues

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Abstract

This manuscript reports the application of in-line dynamic chemical fractionation using a stirred-flow chamber (SFC) for fast characterization of ash-forming metals, *namely*, K, Ca, Na and Mg, and Al as well in woody biomass residues (bark and twigs) and laboratory ashed bark The chemical fractionation procedure is harnessed to discriminate with minimal operational maintenance those target species associated to the water-soluble, the exchangeable and the acid-soluble fractions as a result of their varying occurrence in the biofuels by resorting subsequently to chemicals of increasing leachability. The final goal is to assist in the evaluation of actual pools of ash-forming elements in industrial boilers

The proposed technique is able to handle up to 1.0 g of woody biofuels and provide, as opposed to conventional fuel characterisation, relevant insight into the amount of leachable elements, and real-time quantification of potentially reactive species beside insight into the leaching kinetics of target elements. Screening results can be obtained in less than 3 h instead of more than one week in the batchwise counterpart methods.

For elucidation of metal-biomass/ash associations and investigation of the actual selectivity of extractants, a novel approach has been undertaken by resorting to dynamic fractionation assays in combination of scanning electron microscopy with energy dispersive X-ray fluorescence spectrometry (SEM-EDX) and X-ray diffraction (RXD) assays.

Keywords: Woody biofuels; biomass ash; chemical fractionation; automatic analysis;

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1. INTRODUCTION

Biomass is an important source of renewable energy as well as a vital part of the waste management infrastructure. The use of a great variety of biomass fuels and blends thereof as viable alternatives to fossil fuels for power production around the world has launched the quest of novel methods for reliable prediction of the behaviour of biofuels in power producing installations. In fact, the introduction of biomass fuels might lead to increased corrosion risks of metal parts in furnaces and boilers, fouling in the convection pass where the heat is recovered from flue gases, bed sintering in fluidized bed boilers and slagging of ash in the furnace [1-6]. The immediate consequence is the deterioration of the efficiency of combustion devices since steam temperatures have to remain much lower in contrast to conventional coal firing. It is thus of utmost relevance to get insight into fuel characteristics when firing biomass fuels or new fuel blends so as to optimize the steam temperatures.

In regards to standard fuel assays[3], the so-called proximate analysis determines the moisture, ash and char/volatiles contents for prediction of burning characteristics. The ultimate analysis determines the contents of C, H, O, S, N and Cl for prediction of flue gas composition whilst elemental analysis includes digestion methods for the determination of ash-forming elements for prediction of the ash composition. A standard test method is important to be established for any new, unknown fuel. However, the information retrieved is not sufficient when predicting fuel ash behaviour for potential corrosion risks in combustion devices. This holds true for solid biomass fuels of any kind, whereby advanced fuel characterization methods are to be devised for proper evaluation of the chemical associations in ash forming matter prior to the actual industrial use of such fuels[1,3,5,7].

Partitioning of metal species in solid substrates (e.g., soil, sediment, sludge) into operationally defined forms under the sequential action of given leachants with increasing aggressiveness has been recommended by IUPAC [8,9] for distinguishing various forms of metals according to their mode of occurrence, physico-chemical mobility and potential availability[10-15]. The extractants used in sequential extraction procedures (SEPs) are intended to mimic real scenarios promoting the mobilization of metals associated with certain mineralogical phases and organically-bound metals.

Ash-forming matter in fuels has been investigated for more than two decades by resorting to a three-step SEP involving distilled water, ammonium acetate at neutral pH and hydrochloric acid as extractants. The method was originally developed for the characterization of coal[16], modified for the characterization of biomass fuels[17], and further extended to biomass characterization[1,3,18-21] This method has been harnessed to evaluate how ash-forming elements are bound in the fuel[5,22-25] Different types of ash-forming matter are discriminated

according to their solubility in aqueous solvents. Typical ash-forming components, which are leached out by water include alkali sulfates, carbonates and chlorides. Elements leached out by ammonium acetate are believed to be organically associated via ion exchangeable processes. Hydrochloric acid is leaching carbonates and phosphates of alkaline earth and other metals. Silicates and other minerals remain mainly in the insoluble residue.

Batchwise SEPs have proven however to be tedious and time-consuming. Beside neither are the dynamics of the leaching process of biofuels fully understood, which would be of utmost relevance for optimizing the SEP aimed at reducing extraction times, and most importantly, analysis expenses. Three additional major problems have been also recognized in batchwise steady-state SPEs for partitioning metals in environmental solids and solid wastes, that is, the lack of selectivity of leaching agents (phase overlapping) for releasing metals associated to a discrete solid phase [9,12,13], which is to be influenced by the extractant exposure time; the re-distribution of target species among phases during extraction [9,12,13], and the impossibility to operate under the nominal pH of extractants for well buffered samples, e.g., fly or bottom ashes [26].

In order to speed-up the entire operational procedure, prevent re-distribution phenomena and gain full knowledge on the kinetics of the metal leaching processes the application of automatic dynamic (non-equilibrium) partitioning is herein proposed for determination of K, Na, Ca and Mg in biofuels and ashes thereof and prediction of ash behaviour. In automatic dynamic leaching tests, the solid sample under investigation is loaded in a suitable container, which is exposed continuously to fresh extractant volumes by resorting to flow-based approaches[13,17]. In our case, metered amounts of woody biofuels (twigs and bark) or bark ashes are contained in a stirred-flow chamber (SFC) as a front-end to inductively-coupled plasma atomic emission spectroscopy (ICP-AES) for in-line chemical analysis of the extracts.

Leaching tests, either in manual batchwise or continuous-flow dynamic mode, do not however provide any information as to the actual mobilizable chemical species of the ash-forming matter in biomasses or ashes. To tackle this issue and investigate the actual selectivity (or lack of it) of reagents in the three-step SPE, a novel approach involving the combination of chemical fractionation data with scanning electron microscopy-energy dispersive X-ray fluorescence spectrometry (SEM-EDX) on fuels or ashed fuels[21,28] and X-ray diffraction (RXD) assays of the solid substrates and residues leftover after each step of the dynamic SEP is herein proposed and evaluated.

2. MATERIALS AND METHODS

2.1. Sampling and sample preparation

A ca. 100-year old Scots pine (Pinus Sylvestris L.) was harvested in the municipality of Kronoby (Midwest Finland). The thinnest branches (< 1.0 cm), the so-called twigs, containing both wood and bark, were collected as a whole together with the foliage. In the laboratory, the foliage was separated and the twigs were oven-dried at 105°C until constant weight. Prior to analysis, the biomass samples were grinded to a particle size less than 1 mm in a bench mill and placed in sealed polyethylene bags in dark storage. A second sample, bark, was provided for by a boiler operator in western Finland. This sample was treated in a similar way as the twigs. Particular attention was paid to ensure a representative test portion for automatic SEPs.

Bark and laboratory prepared bark ashes were chosen as model samples for characterization of metal solid phase associations and the potential overlapping of phases in the SEP. Bark ash was obtained as per BS EN 14775:2009 [29] by heating 1 g of bark in a porcelain crucible from 250°C (heating rate at 5 K min^{-1} and held for 60 min) to 550°C (heating rate at 5K min^{-1} and held for 120 min). The amount of ash formed was 4.99 ± 0.30 wt-% of the original dry bark, which is in good agreement with earlier reports in the literature[19].

2.2. Characterization of samples

The topology of bark ash particles as well as of the solid residues remaining after each extraction step was examined by Scanning Electron Microscopy (SEM). In addition, the distribution of target elements in the sample structures was explored using Energy Dispersive X-ray Fluorescence Spectrometry (EDX). The main crystalline phases leftover after each extraction step were identified by qualitative X-ray powder diffractometry (XRD) using an X-ray powder diffractometer (Siemens D5000, Washington DC, USA) operating with Cu K_α radiation and a scintillation detector. The identification of compounds was performed through comparison with standard reference patterns.

Raw samples of biomass fuels and ashes (0.5 g bark, 0.15 g twigs and 0.1 g bark ash), and residues leftover after dynamic fractionation were digested in a closed-vessel microwave digestion system (MLS-1200 Mega, Milestone, Italy) for determination of total element concentrations and immobilized metal fractions, respectively. To this end, samples were carefully transferred to PTFE vessels to which 5 mL of concentrated HNO_3 (65% w/w) and 3 mL of concentrated H_2O_2 (30% w/w) were added. A first microwave digestion program composed of seven consecutive steps was utilized as per literature recommendations[30]. After cooling down to room temperature, samples were subjected to a second digestion protocol

with further addition of 2 mL of HNO_3 and 1 mL of H_2O_2[30]. Digests were finally diluted to 50 mL with Milli-Q water.

2.3. Instrumentation and automatic analytical procedure

The automatic setup (FIAlab-3000, FIAlab, Bellevue, US) was composed of a 6-port multiposition selection valve and a 30,000-step syringe pump (Cavro, Sunnyvale, USA) with a capacity of 5 mL (see Fig. 1) for handling of leaching reagents and delivery of well-defined volumes of extractants to the solid sample as contained in a stirred flow chamber (SFC, see below). The system is fully computer controlled via the user-friendly FIAlab associated software for Windows 5.0. The central port of the valve was connected to the pump via a holding coil, which consisted of a 3.0-m long polytetrafluoroethylene tubing (1.5 mm i.d.) with an internal volume of ca. 5.3 mL. The outlets of the valve were connected to the extractant reservoirs, SFC or waste using PTFE tubing of 1.5 mm i.d.

The automatic analytical sequence comprised the consecutive aspiration of the individual extractants from different external ports of the valve which sequentially are exposed to biomass or ash samples. For evaluation of the water soluble fraction, the syringe pump was programmed to aspirate 4900 µL of distilled water (port 2) whereupon the flow was reversed and the extractant perfused the solid sample at 2.5 mL min^{-1}. The main leachate stream was subjected to real-time pH detection using a PASPORT pH sensor (PS-2102, Pasco, Roseville, USA) (see Fig. 1) and to continuous sampling at 0.17 mL/min (using a peristaltic pump, PP1) for in-line monitoring of leachable ash-forming matter by ICP-AES. This secondary stream merged downstream with a steady flow of 2% (v/v) HNO_3 provided by the peristaltic pump PP2, which fed the ICP-AES at 1.5 mL/min. Afterwards, the syringe pump was refilled with distilled water from the external reservoir at the maximum allowed flow rate for the given syringe size (21 mL min^{-1}). This automatic procedure was repeated a number of cycles until the ICP-AES readouts reached the baseline for the overall elements. The same procedure was undertaken for the ammonium acetate fraction using an extractant volume of 118 mL. Prior to changing to the last fraction, the content of the stirred reactor was rinsed with ca. 30 mL of distilled water. Then, the entire flow manifold components (SFC, holding coil and HCl reservoir) were placed in a thermostatic bath to assure the acid extraction step to occur at 70 ± 5 °C. Experimental results revealed that overall acid soluble metals were released in less than 118 mL of 1.0 mol L^{-1} HCl at 70 °C. Afterward, a second washing step with ca. 30 mL of distilled water was implemented before collection of the biomass or ash residue for determination of the pool of immobile metal species.

The leachate stream was analyzed with a Perkin-Elmer Optima 5300 DV inductively plasma atomic emission spectrometer (Perkin Elmer, MA, USA) equipped with a GemTip Cross-flow pneumatic nebulizer (Perkin Elmer) utilizing manufacturer's recommendations[24]. The instrument readouts were recorded every minute for construction of the leaching profiles. A matrix-matched external calibration procedure with multi-elemental standard solutions was used for quantification of target elements in the leachates employing a continuous-flow pumping system capitalized on PP1.

2.4. Characterization of the stirred flow chamber as extraction reactor

The SFC constructed from borosilicate glass with a nominal volume of ca. 15 mL was designed to foster extractants to flow sequentially through the woody biomass or bark ash for leaching of metals associated to nominal phases. A weighed dry solid sample (500 mg for bark; 150 mg for twigs and 100 mg for bark ash, as per their ash-forming matter content and swelling behaviour) was transferred to the SFC together with a small magnetic bar (1 cm long). A rubber gasket was placed on top of the SFC followed by a nylon filter (GE Osmonics Labstore, USA) of 0.45 µm pore size and 47 mm diameter to retain suspended particles. The SFC is completed with a second rubber gasket and the cover on top of the chamber extraction whereupon the overall components were tightly clamped. The entire reactor was placed on a magnetic stirrer and the sample was mechanically agitated in the extraction milieu at 500 rpm**.** The inlet of the SFC is connected to the valve of the flow manifold and the outlet to PP1 and pH sensor via a T-confluence (see Fig 1).

In this work we explored the transient concentrations of target species and residence time distribution (RTD) in the SFC reactor. RTD is a relevant parameter for investigation of the mixing and flow behaviour within continuous stirred-tank reactors[31]. In continuously operated SFC reactors, experimental conditions (e.g., design of magnetic stir bar, agitation speed and configuration of the reactor itself) are to be optimized to ensure uniform mixing of the SFC content. This would thus imply that the composition of the outlet stream is identical to the bulk SFC contents.

For estimation of RTD, a single inert salt, namely, 1.0 mol L^{-1} $Ca(NO_3)_2$, was used as a tracer. The stirring and hydrodynamic conditions were matched to those of the dynamic SEP for advanced biomass characterization. A 1-mL pulse of tracer was added to the SFC as fast as possible followed by carrier pumped at 2.5 ml min^{-1}. The effluent was automatically injected into ICP-AES for measurement of the transient signal of calcium from which the RTD function (so-called E(t)) [31] was calculated.

3. RESULTS AND DISCUSSION

The characterization of phase composition of a given solid biomass fuel and ash thereof should be regarded as a crucial step in determining the actual applicability of the fuel. To date, batchwise chemical fractionation has been harnessed to advanced fuel and ash characterization [1,3,5,17,20,22-24,32,33]. However, there is still a quest of seeking novel fractionation methods for metal partitioning in biomass able to overcome the number of shortcomings of manual methods pinpointed in the introduction. Dynamic flow-through column-based methods capitalized on continuous pumping of extractant through the solid material have been yesteryears the method of choice to improve the analytical performance of classical partitioning methods of trace elements in environmental solids[10-13,27,34-43], yet the minute amounts of solid samples usually handled (≤200 mg)[34,35,37-39,42,43] to circumvent backpressure effects do not assure sample representativeness for non-homogeneous samples, making the actual applicability of these methods to real-sample assays debatable. To tackle this shortcoming, which also may occur in analyses of biofuels, we herein propose a flow-based configuration incorporating an SFC as the extraction reactor assembly for accommodation of up to 1.0 g of woody biomass on the basis of the swelling properties of the biofuels and expected level of metal content.

For characterization of the continuous SFC reactor, the RTD was calculated using a tracer as described in the experimental section**.** The RTD function (E(t)) (see Fig. 2) assists in elucidating any mixing problems, such as bypassing (or channeling) and stagnant zones in the reactor, on the basis of the E(t) pattern[31]. The inverse of the intercept of the exponential decay curve with Y-axis coincides with the average residence time of the tracer (and later leached elements) within the SFC reactor. In our particular case, the average residence time was estimated to be 5 min, which demonstrated operation of SFC at 500 rpm under well-mixed conditions with an effective mixing volume of 12.5 mL. The knowledge of the behaviour of the SFC reactor under continuous stirring conditions provides a good basis for scaling-up of dynamic chemical fractionation tests for inhomogeneous samples

3.1. Dynamic flow-through fractionation assays of biomass fuels and ashes

The release of ash-forming matter in biomass fuels is a function of the biomass type and the source thereof but the sampling, the storage and the metal-solid phase associations as well [3,5,23]. Accurate control of the pH of extracts is needed in SEPs for discrimination of binding strengths of metals to biofuel or ash matrixes. Coupling of dynamic SEP with in-line pH measurements and ICP-AES detection has been in this work utilized for real-time monitoring of both the leachate pH and the released ash-forming elements (see Fig. 3 and Fig. S1). For the

water-soluble fraction of woody biomass fuels (bark, twigs) the initial acid pH (pH<5) is a consequence of the release of water-soluble organic acids[3] and acid phosphate salts as well. On the contrary, the release of the water soluble fraction in bark ash at the nominal extractant pH is offset by the intrinsic buffer capacity of the water leachates at pH>10 (see Fig. S1), because of the release of water soluble alkali salts, e.g., carbonates. Upon changing the extractant composition to ammonium acetate, the pH profile of bark ash extracts gradually decreases until approaching to the nominal pH (ca. 7.0) in about 40 min. For biomass (bark and twigs) samples, the extractant nominal pH is rapidly reached in ca. 10 min.

The plot of the concentrations of leached metals (obtained in-line by ICP-AES) against extraction time (Fig. 4) provides a good account of the maximum pools of mobilizable metals because of the exhaustive extraction nature of dynamic fractionation, and of the binding strengths of metals to nominal phases through evaluation of peak widths. In addition, the amount of leachable elements might be readily quantified as the product of the area of the extraction profiles in Fig. 4 and the extractant flow rate. The overlay plots of leachate pH profiles with real-time elemental assays by ICP-AES (Fig. 3) offer detailed insight into the actual pH at which metal-biofuel associations are mobilized and the efficiency of the extractant as well.

Potassium is the most important ash forming element contributing to lower process performance in furnaces and fluidized-bed boilers. Potassium occurs in woody biomass in varied chemical forms of different reactivity, including ionic forms in the sap of plants or associated to ion-exchangeable organic moieties. The less mobile fraction of potassium is frequently found as clay contaminations (see SEM-EDX and XRD results in the next section) of the fuel, the magnitude of this pool being determined by analyzing the non-soluble residual fraction.

The results of automatic leaching tests with bark, twigs and bark ash for Na, K, Mg, Ca and Al and P are summarized in Table 1. It can be seen that over 79 and 88% of potassium in the bark and twigs analyzed (1094±72 mg/kg and 2344±72 mg/kg, respectively) are found in the water leachable fraction, most likely associated to chloride (and additionally to phosphate or sulphate[5]), which participates in the lively chemistry of gaseous alkali forms in flue gases. Likewise, more than 60% of leachable potassium in bark ash is released with water using a dynamic extraction mode. The water soluble potassium is leached in less than 30 min in the overall biofuels (see Fig. 4) by resorting to dynamic SFC-based SPEs rather than 24 h as recommended in batchwise fractionation methods. This also holds true for sodium, yet the concentrations in bark and twigs are 20 and 30-fold lower, respectively, in the water-soluble fraction. In bark ash, sodium was observed in the exchangeable fraction as well because of changes in mineralogical phases in the course of ash formation.

Calcium is the element in the highest concentration in bark fuels. Our results confirmed a 15-fold increase in the overall leached calcium in bark as compared to twigs. As opposed to alkalis and magnesium (see Fig. 4 and Table 1), calcium is mostly leached in the ammonium acetate (36% in bark and 38% in twigs) and acid (55% in bark and 47% in twigs) fractions. This is a consequence of the fact that calcium is associated to carboxylic acids of cell structures, via ion-exchange reactions. Calcium was however leached out slower in the exchangeable fraction of bark and bark ash than that of twigs as revealed by broader peak shapes in the extraction profiles (see Fig. 4). As a result, large amounts of extractant for quantitative release of calcium might be called for.

In contrast to twigs, wherein a significant amount of water-soluble aluminum salts are encountered, aluminum in bark is leached out to a large extent (70%) in the acid-soluble fraction, being indicative of the presence of aluminum as bark impurities.

3.2. Elucidation of metal-biofuel and metal-ash solid phase associations

The automatic dynamic SEP has been proven suitable for discrimination of binding mechanisms and leaching kinetics of potentially corrosive elements in woody biomass fuels and a laboratory prepared ash thereof. Yet, chemical fractionation cannot be resorted to identify the actual mode of element occurrence. For elucidation of metal-biofuel/ash associations and investigation of the selectivity of SEP extractants the interplay of chemical fractionation and SEM-EDX and XRD assays is of utmost relevance. To this end, SEM-EDX and XRD were resorted to the analysis of bark ash in residues leftover after each step of the SEP.

EDX mappings of raw bark ash and residues are illustrated in Fig 5. The occurrence of an element is shown as bright spots whereby the brighter the spot the higher is the concentration of target element. EDX mappings of raw bark ash revealed good qualitative correlations of potassium with aluminum and phosphorus. The latter is however not detected after water extraction, thereby suggesting the presence of potassium phosphates in the ash matrix. Identification of associations of potassium with aluminum silicates in mappings after the first and second fractionation steps (immobilized potassium phases) was corroborated by XRD with the finding of phlogopite and sanidine (Fig 6). Notwithstanding the fact that the detection limit of crystalline phases in XRD is ca. 2 wt-% and that the composition of amorphous phases cannot be identified, XRD assays were harnessed to explore the selectivity of leaching reagents. Both calcite and anhydrite ($CaSO_4$) were entirely released in the ammonium acetate fraction (absence in Fig. 6), whereby the content of ion-exchangeable elements in bark ash is overestimated and the acid-soluble fraction underestimated. This result is in accordance with earlier reports of the dissolution of calcite and gypsum with ammonium acetate [22,44].

The crystalline inorganic matter with elevated calcium content in the raw bark and dissolved with ammonium acetate was identified as whewellite ($CaC_2O_4 \cdot H_2O$) over a structured background in XRD. It should be borne in mind that calcium oxalate decomposes to carbonate during combustion of biomass at temperatures above 400 ºC [45], and hence, this crystalline phase is not observed in the XRD of ashes.

3.3 Automatic analysis of biomass fuels and ashes

The analytical performance of the automatic flow setup for dynamic SEP has been assessed through the analysis of Na, K, Mg, Ca and Al in woody biomass residues described above. Triplicate fractionation assays of overall samples were undertaken with ease with minimum analyst intervention. Mass balance validation involving quantification of leachable and immobilizable forms of ash-forming elements and comparison with total element content as determined by microwave digestion was utilized as a quality control tool so as to demonstrate that the automatic method herein proposed renders unbiased results. In fact, relative recoveries for the suite of elements analyzed in-line ranged from 97 to 109% regardless of the element, sample and fraction type (see Table 1). The *t*-tests of comparison of means for the overall assays between the sum of leachable and immobilized elements and the total metal content confirmed the inexistence of significant differences at the 0.05 significance level. A good agreement was also found between total amounts of elements in raw bark and bark ash.

In conventional batchwise SEPs of biomass fuels single fractionation assays (without replicates) are often reported in the literature[22,23], with analysis of merely the liquid portion of the sample as a consequence of time and cost constraints. Therefore, the methods cannot be validated in terms of uncertainty and bias of experimental results. In fact, researchers have reported difficulties in obtaining good mass balances[20] because of the non-homogeneity of fuel samples and the large number of manual operations involved requiring trained and educated personnel with laboratory skills.. The largest problem has been encountered with analytical constraints with contaminated fuels containing a substantial amount of silicon. In addition, manual SEPs for partitioning of ash-forming matter in biofuels usually last more than one week [3,17,19,22,23,32,33].

On the contrary, the automatic dynamic method is proven less cumbersome, more reliable (replicate measurements are easily performed) and amenable to substantially improve the sample throughput. When applying the developed SEP method to other type of solid fuels the operating parameters should be optimized each time. Because amounts of biomass up to 1 g

might be handled in the SFC special care should be taken to ensure representative sample for analysis. Different biosamples may also require different solid to liquid ratio for exhaustive dynamic SEP. The entire analysis of the water soluble and exchangeable fractions including leaching and ICP-AES measurements takes ca. 30 and 60 min, respectively, even for the high calcium containing exchangeable fraction in bark. In contrast, extraction of the two more reactive phases in conventional methods last 24 and 72 h, respectively, without accounting for phase separation and measurement of the metal content in leachates by atomic spectrometric techniques. Experimental results of the application of the batchwise SEP[23] for twigs, bark and bark ash are also compiled in Table 1 for comparison. It should be noted that recoveries of some elements (e.g., Na in bark and Mg and Al in twigs) are significantly lower than 100 % (*namely,* 76-85%) because of losses of elements during manual operations.

4. CONCLUSION

This paper reports the application of a dynamic flow-through chemical fractionation test involving hyphenation of SFC reactor with ICP-AES for advanced characterization of woody biomass fuels (viz., clean wood and bark) as well as a laboratory-ashed bark. The automatic method is able to delivery fast results which might be of potential interest to industry. In fact, extraction and quantification of leachable alkali and alkaline earth metals take less than 3 hours in contrast to more than one week in the manual counterparts.

Experimental results showed a good closure of mass balance over the leaching steps and comparable results with the batchwise procedure. Future research should be devoted to explore the automatic flow system for expedient investigation of other ash-forming elements and fuel groups.

Besides the identification of the actual mode of element occurrence and elucidation of metal-solid phase associations, the combination of dynamic SEPs with SEM-EDX and XRD analysis lead us to investigate the selectivity of extractants. We conclude that the reagent in the second step should be further optimized to prevent the overestimation of the ion-exchangeable fraction of calcium as a consequence of the release in this fraction of calcite and anhydrite.

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384 **Table 1** Leachable amounts of ash-forming elements and Al in bark, twigs and bark ash by resorting to automatic dynamic fractionation and
385 comparison with batchwise equilibrium extraction

element	fraction I (H_2O)		fraction II (CH_3COONH_4)		fraction III (HCl)		total leachable metal [a]		residue		total [b]	recovery [c]	
	automatic	batchwise	automatic	batchwise	automatic	batchwise	automatic	batchwise	automatic	batchwise	microwave d	automatic	batchwise
	mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}	%	
bark													
Na	85 ± 2	76 ± 2	nd	nd	nd	nd	85 ± 2	76 ± 2	174 ± 4	119 ± 10	258 ± 10	100	76
K	1094 ± 72	747 ± 45	41 ± 4	309 ± 8	29 ± 8	79 ± 3	1164 ± 60	1135 ± 38	252 ± 6	222 ± 5	1386 ± 32	102	98
Mg	369 ± 10	296 ± 21	234 ± 22	267 ± 12	39 ± 2	55 ± 2	642 ± 18	618 ± 35	83 ± 3	97 ± 8	720 ± 13	99	100
Ca	571 ± 37	406 ± 4	2429 ± 117	858 ± 18	3725 ± 113	4708 ± 137	6725 ± 169	5972 ± 126	300 ± 123	793 ± 46	7057 ± 59	99	96
Al	72 ± 2	66 ± 3	40.8 ± 0.8	6.7 ± 0.1	260 ± 7	185 ± 2	373 ± 8	258 ± 4	511 ± 15	616 ± 3	888 ± 6	99	98
twigs													
Na	11.3 ± 0.3	19 ± 2	nd	nd	nd	nd	11.3 ± 0.3	19 ± 2	153 ± 3	143 ± 13	167 ± 5	98	97
K	2344 ± 32	1975 ± 113	nd	nd	nd	nd	2344 ± 32	1975 ± 113	321 ± 2	365 ± 12	2667 ± 35	100	88
Mg	358 ± 18	320 ± 26	337 ± 7	234 ± 26	nd	nd	695 ± 25	554 ± 52	25.4 ± 0.9	54 ± 8	716 ± 9	101	85
Ca	409 ± 16	223 ± 11	1153 ± 36	586 ± 52	1507 ± 20	1763 ± 47	3069 ± 61	2572 ± 37	1455 ± 30	1725 ± 73	4636 ± 24	97	93
Al	197 ±15	86 ± 1	72 ± 2	36 ± 2	137 ± 6	88 ± 6	406 ± 24	210 ± 4	54 ± 2	149 ± 2	421 ± 14	109	85
bark ash [d]													
Na	23.0 ± 0.9	22.4 ± 0.8	10.3 ± 0.7	10.1 ± 0.4	nd	nd	33.3 ± 0.3	32 ± 1	99 ± 2	95 ± 5	134 ± 3	98	95
K	807 ± 27	774 ± 24	104 ± 5	104 ± 5	201 ± 9	204 ± 14	1111 ± 38	1082 ± 42	190 ± 9	156 ± 12	1324 ± 19	98	93
Mg	39 ± 3	38 ± 2	484 ± 10	481 ± 9	118 ± 11	132 ± 3	641 ± 8	651 ± 11	123 ± 7	106 ± 6	770 ± 11	99	98
Ca	382 ± 4	396 ± 2	5724 ± 38	5854 ± 97	375 ± 22	364 ± 12	6481 ± 45	6614 ± 83	242 ± 8	46 .0 ± 0.8	6741 ± 146	99	98
Al	196 ± 3	161 ± 5	6.4 ± 0.4	9.0 ± 0.5	434 ± 4	378 ± 4	636 ± 8	548 ± 10	182 ± 8	257 ± 32	821 ± 17	99	98

Results are expressed as the mean of three replicates ± standard deviation

nd: not detected

[a] summation of leachable concentrations in fractions I, II, and III

[b] total metal content in the original sample

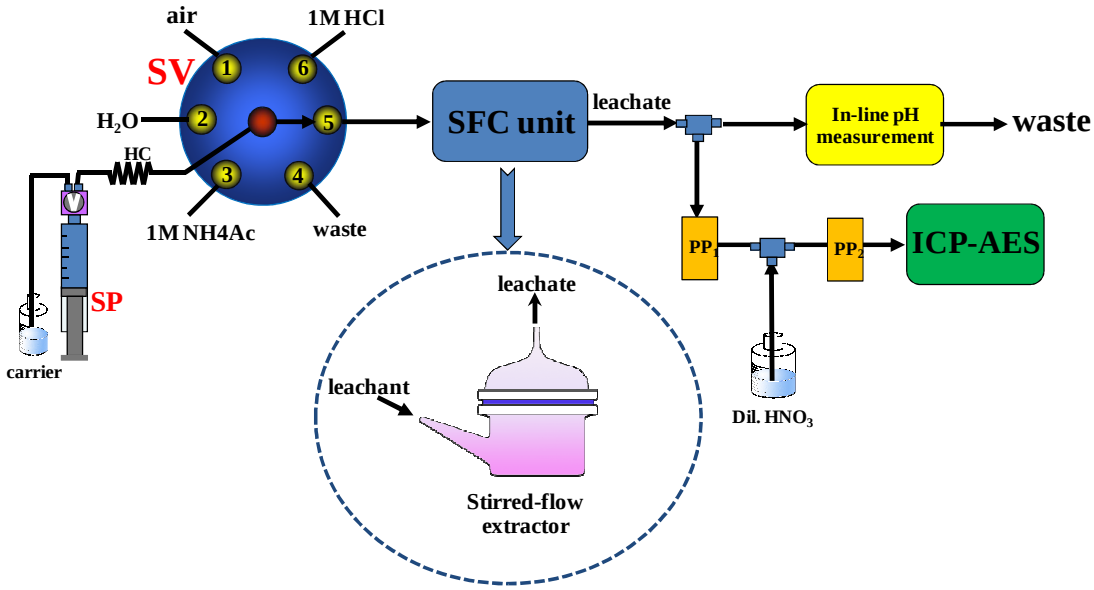
[c] calculated as the ratio of mass balance (step I+II+III+ residual) to total metal content.

386

387

FIGURE CAPTIONS

Figure 1. Sketch of the automated flow system for dynamic chemical fractionation



394 **FIGURE 2.** Plot of residence time distribution (E) versus time in the stirred-flow cell
395 reactor

396

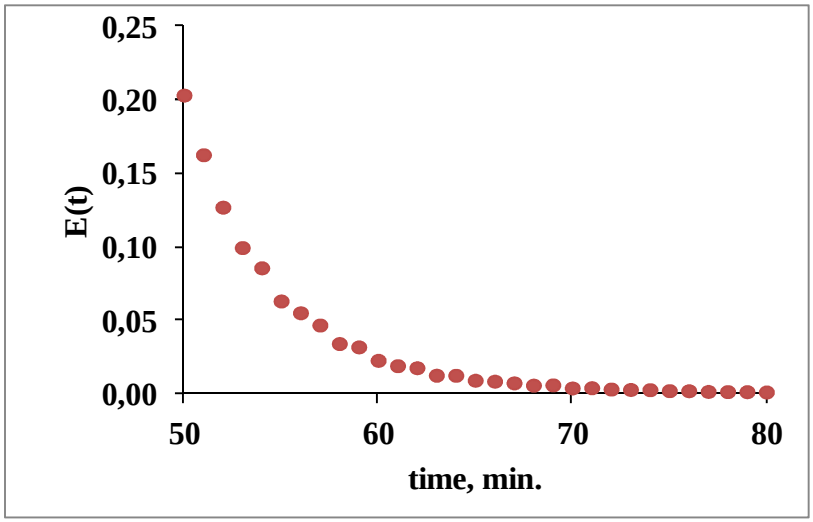


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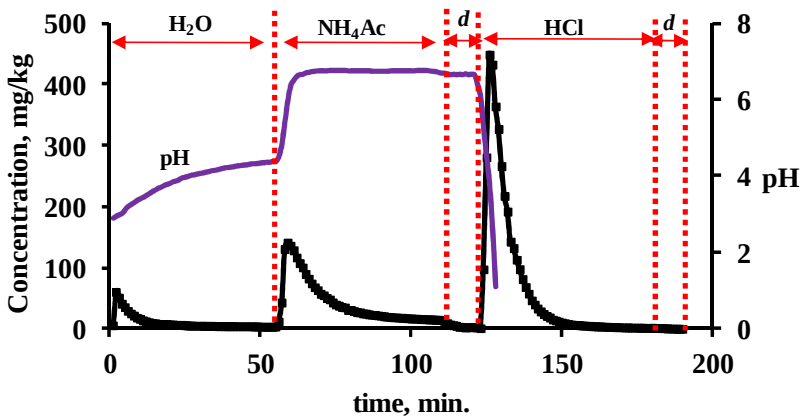
FIGURE 3. Overlay of in-line leachate pH patterns with extraction profiles of calcium in bark as obtained by dynamic chemical fractionation.



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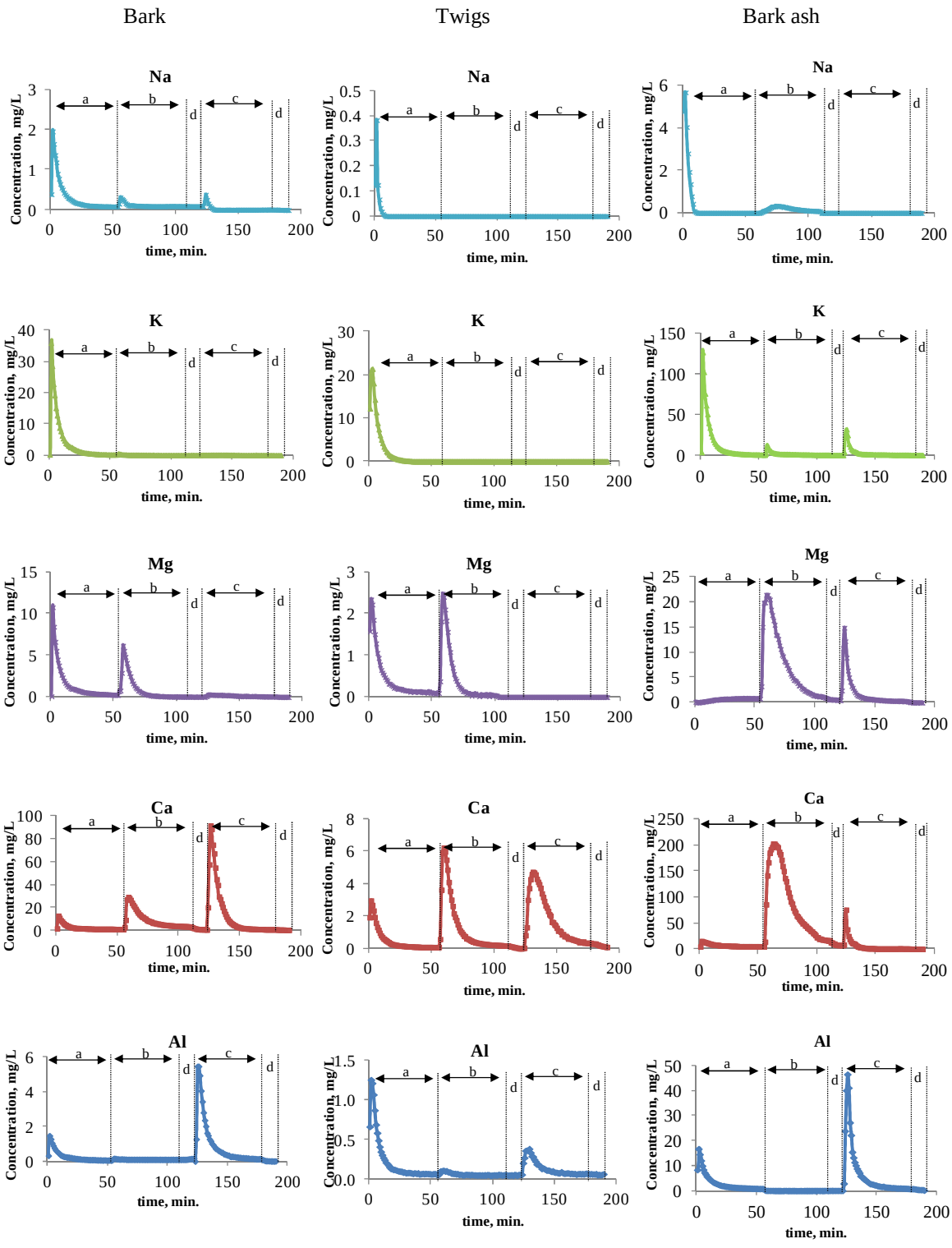
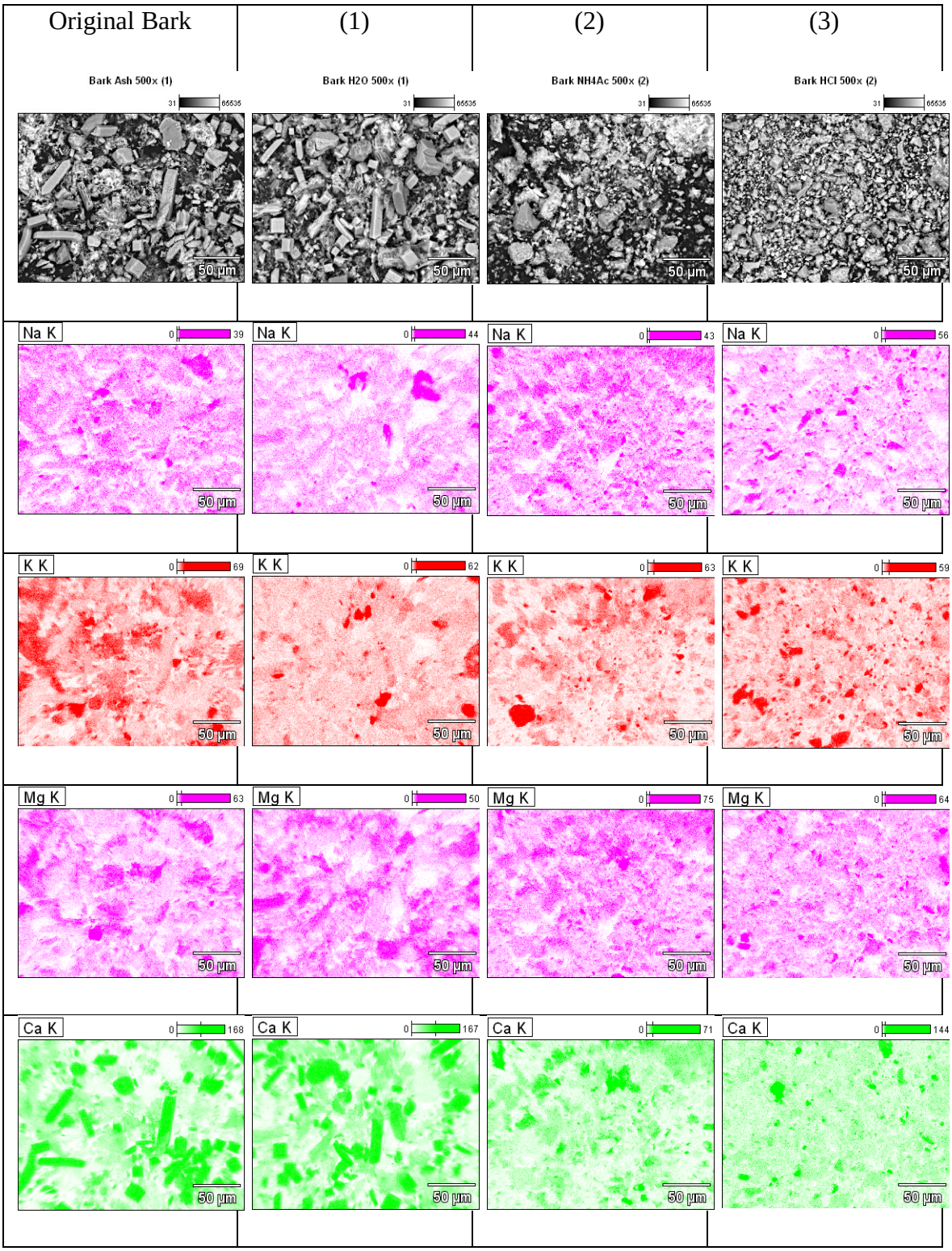
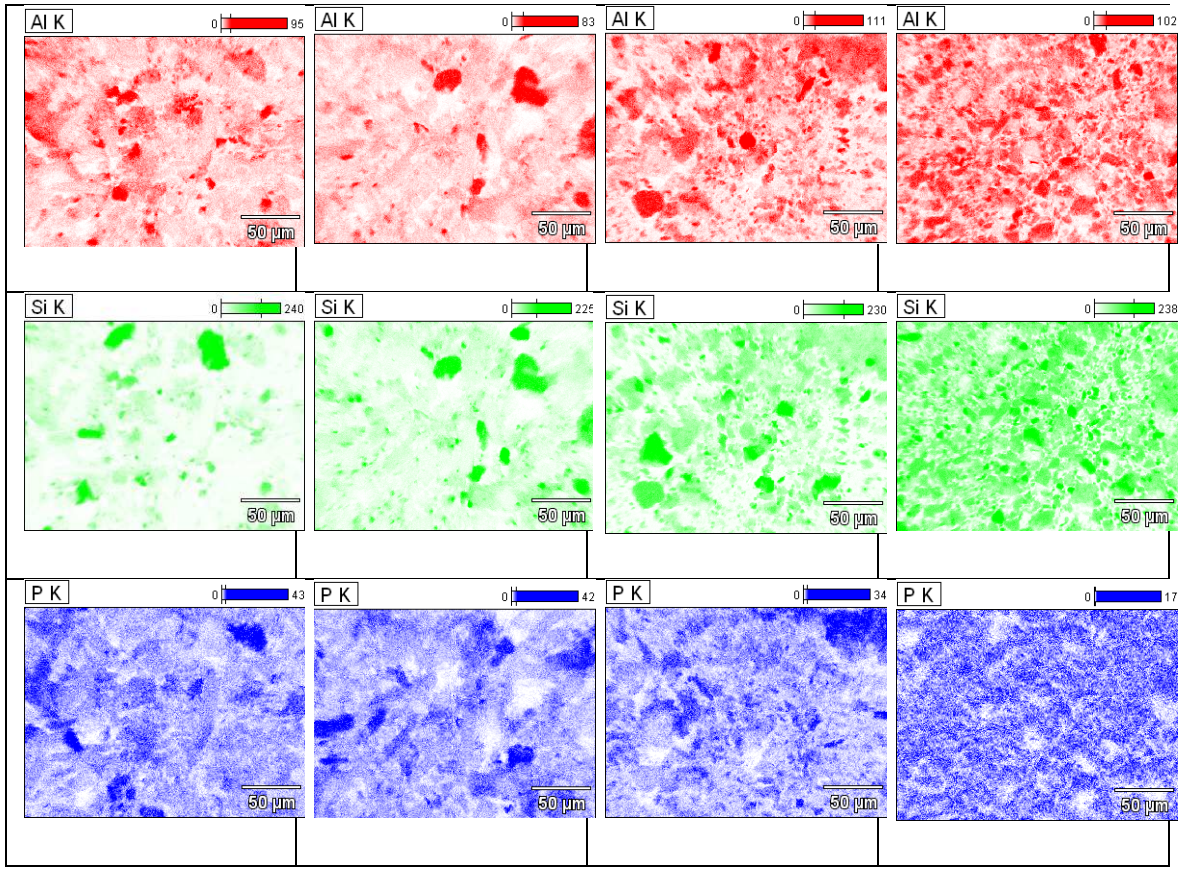


410 **FIGURE 4** Extraction profiles of ash-forming elements in bark, twigs and bark ash
411 samples using SFC-based chemical fractionation: a) Water soluble fraction, b) Ion
412 exchangeable fraction, c) Acid soluble fraction, d) Water cleaning fraction.

413

FIGURE 5. EDX mappings of target elements in raw bark ash and residues leftover after extraction with water (1), water + ammonium acetate (2) and water + ammonium acetate + hydrochloric acid (3).




Al K
0
95
50 µm
Al K
0
83
50 µm
Al K
0
111
50 µm
Al K
0
102
50 µm
Si K
0
240
50 µm
Si K
0
225
50 µm
Si K
0
230
50 µm
Si K
0
238
50 µm
P K
0
43
50 µm
P K
0
42
50 µm
P K
0
34
50 µm
P K
0
17
50 µm

418

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420 **FIGURE 6** XRD analysis of bark ash residues leftover after water + ammonium acetate dynamic extractions

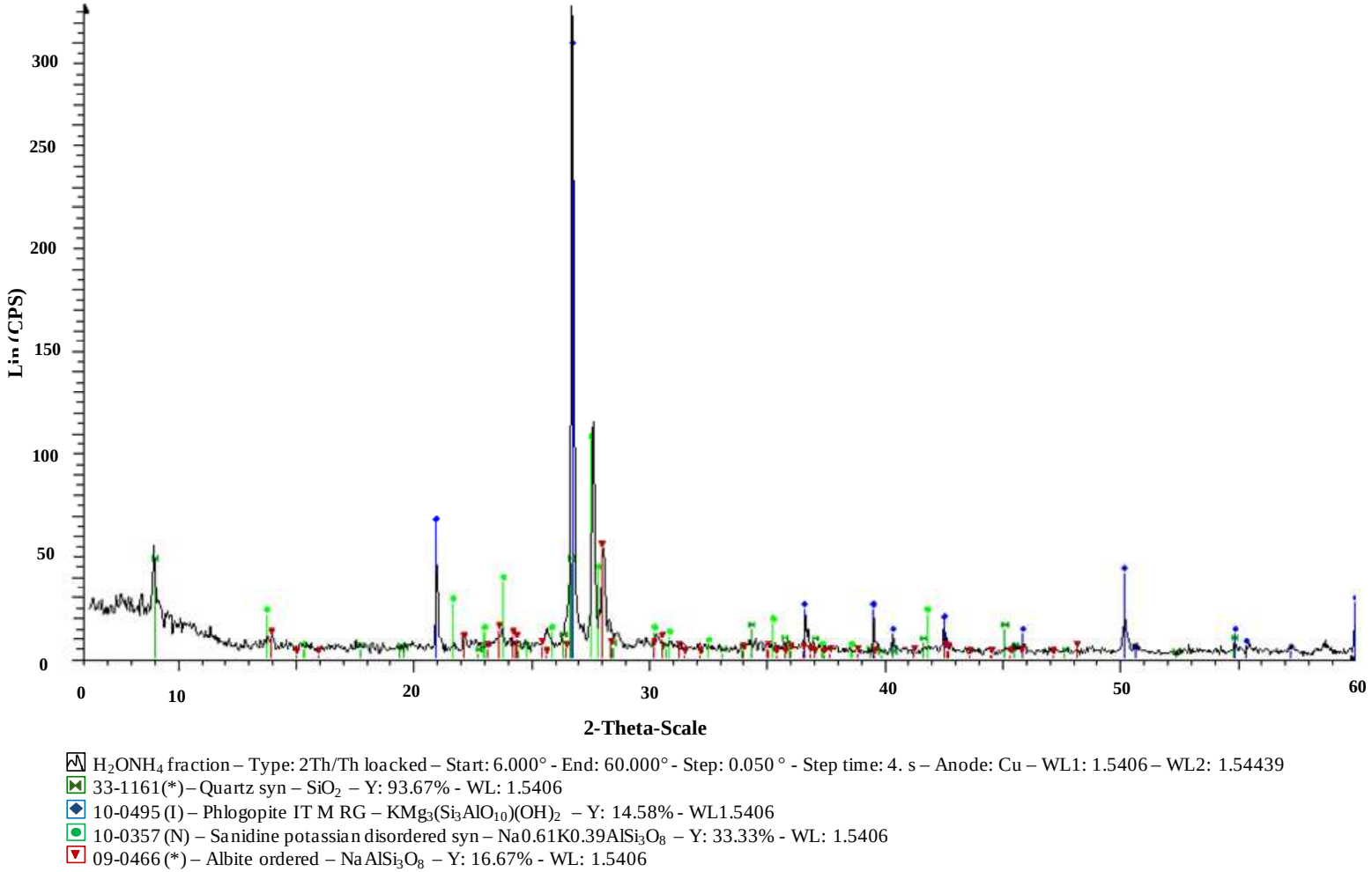

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FIGURE CAPTIONS

Figure 1. Sketch of the automatic flow system for dynamic chemical fractionation. SP: Syringe pump. SV: Selection Valve. PP: Peristaltic pump. SFC: Stirred flow chamber. ICP-AES: inductively coupled plasma-atomic emission spectrometer.

Figure 2. Plot of residence time distribution (E) versus time in the stirred-flow cell reactor.

Figure 3. Overlay of in-line leachate pH patterns with extraction profiles of calcium in bark as obtained by dynamic chemical fractionation.

Figure 4. Extraction profiles of ash-forming elements in bark, twigs and bark ash samples using SFC-based chemical fractionation: a) Water soluble fraction, b) Ion-exchangeable fraction, c) Acid soluble fraction, d) Water cleaning fraction.

Figure 5. EDX mappings of target elements in raw bark ash and residues leftover after extraction with water (1), water + ammonium acetate (2) and water + ammonium acetate + hydrochloric acid (3).

Figure 6. XRD analysis of bark ash residues leftover after water + ammonium acetate dynamic extractions.

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CHAPTER 7

Online hyphenation of multimodal microsolid phase extraction involving renewable molecularly imprinted and reversed-phase sorbents to liquid chromatography for automatic multiresidue assays

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Online Hyphenation of Multimodal Microsolid Phase Extraction Involving Renewable Molecularly Imprinted and Reversed-Phase Sorbents to Liquid Chromatography for Automatic Multiresidue Assays

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Molecular imprinted polymers (MIP) have recently drawn much attention as highly selective solid-phase materials for handling and isolation of organic pollutants in complex matrices. Because of the impaired retention capacity for target species as compared with reversed-phase materials and irreversible sorption of interfering compounds by nonspecific interactions, the implementation of MIP-based solid-phase reactors as permanent components in automatic flow-systems has not received widespread acceptance as of yet. To tackle this limitation, a dynamic microscale solid phase extraction (*μ*SPE) method capitalizing on the principle of programmable flow and bead injection analysis is herein proposed as a front end to liquid chromatography for multiresidue assays. It involves in-line renewable tandem-SPE microcolumns composed of molecularly imprinted polymers and copolymeric *N*-vinylpyrrolidone/divinylbenzene beads integrated within the flow network for multimodal extraction. Chlorotriazine herbicides (namely, atrazine, simazine, propazine) and principal degradation products thereof (namely, deisopropylatrazine and deethylatrazine) were selected as model analytes. The effect of several parameters, including the dimensions and chemical composition of the sorptive microcolumns, the sample loading flow rate, the type and volume of eluent, the interface with liquid chromatography (LC), and the disposable nature of the column on the analytical performance were investigated in detail. The assembled flow setup features appropriate removal of interfering organic species via solvent switch with toluene, the circumvention of analyte band-broadening in LC by in-line merging of the eluate with a water stream, and the transfer of the overall analyte-containing eluate into the LC. For 10-mL sample percolation, limits of detection (S/N = 3) of 0.02−0.04 ng mL^{-1}, limits of quantification (S/N = 10) of 0.07−0.12 ng mL^{-1}, absolute recovery percentages >79%, precision within 1.4−5.5%, and enrichment factors of 46−49 were obtained for the suite of assayed herbicides. The multimodal *μ*SPE method with renewable beads was applied to the multiresidue determination of the target herbicides in crude soil extracts and untreated environmental waters at concentration levels below those endorsed by the current EU Water Framework Directives following appropriate sample preconcentration and/or cleanup.

Multiresidue determination of organic contaminants in environmental matrices does pose several challenges to the analyst as a result of the low concentration levels of target species and the concomitant existence of matrix interferences. Therefore, appropriate sample processing schemes are indispensable for removing interfering components while improving the detection capability of the analyte by application of preconcentration schemes prior to chromatographic separations.[1−3] Different formats of solid-phase extraction (SPE) or capillary sorbent microextraction, also implemented in a mechanized flow-based mode,[4−6] have been proposed and exploited over the past years for effective separation and preconcentration of organic pollutants in real-life samples.[2,3,7] Nonselective reversed-phase sorbent materials with tailored polarity are still the common choice for routine processing of environmental matrices.[1,2] Yet, interfering substances might be retained onto the solid surfaces and eluted concomitantly with the analytes. Although chromatographic methods (e.g., multidimensional chromatography) have excellent peak capacity, the quality of the analytical results and lifetime of chromatographic columns might be severely deteriorated whenever matrix effects are not appropriately overcome.

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Immunoextraction sorbents[8] and man-tailored biomimetic material[9–13] are currently regarded as appealing SPE alternatives for selective isolation and/or preconcentration of target species prior to chromatographic separations. Molecularly imprinted polymers (MIP) are garnering increasing interest as compared to their biological counterparts for emulating molecular recognition as a result of the improved chemical stability and less stringent demands as to the reaction conditions in terms of pH and temperature.[10,12] Notwithstanding of the fact that the covalent or semicovalent approaches for MIP synthesis foster strong interactions of the analyte with functional monomers, the noncovalent approach is by far the most commonly exploited for generation of imprinting binding sites because of its universal applicability, faster sorption kinetics, and operational simplicity.[10,14] Ideally, raw samples and crude extracts could be directly processed on the basis of a selective uptake of analytes via imprinted binding sites. Experimental results, however, revealed that nonspecific interactions of matrix components with the copolymer matrix or residual monomers at the surface might hinder the efficient binding of target species to the specific recognition cavities with the consequent deterioration of the extraction recoveries and sorbent lifetime.[15−17] Matrix effects could be alleviated partially whenever the sample is appropriately buffered prior to MIP percolation[13] and optimized rinsing protocols with a given sequence of solvents are implemented within the analytical procedure.[10,13,17] Loading of the sample onto a nonimprinted polymer and MIP in series has been also proven suitable to minimize the nonspecific sorption of interfering species.[18,19]

The multistage nature of molecularly imprinted-SPE (MISPE) procedures, encompassing an additional solvent switch when processing water samples with noncovalent MIP,[13] made the automation of the overall protocol via flow-based approaches[5,20,21] cumbersome. Further difficulties are associated with the incompatibility of organic solvents inherent to MISPE of water matrices with the components of the flow network and HPLC separation. Moreover, a progressively tighter packing of the polymer is frequently observed whenever the MIP-containing packed reactor is utilized as an integral constituent of the flow network[22] and thus reused for a given number of assay cycles, which gives rise to the deterioration of the retention efficiency of target species.

To tackle the above limitations, we herein propose the exploitation of microscale SPE with renewable surfaces in flow systems, termed bead injection (BI), that automates the packing and retrieval of the sorbent material within the flow conduits in each individual assay.[23,24] To the best of our knowledge, no miniaturized BI−MISPE procedure hyphenated to chromatographic separations for multiresidue analysis has been reported as of yet. As a result of the low binding capacity of MIP by either reversed-phase or normal-phase interactions as compared to conventional nonspecific sorbent materials (e.g., octadecyl-chemically modified silicagel or (polar-enhanced) copolymeric phases) for uptake of target species,[25,26] an automatic tandem-column multimodal-BI approach combining water-compatible MIP and reversed-phase mixed-mode sorption prior to online LC separation has been developed and validated for selective preconcentration and determination of priority environmental pollutants at concentration levels below those endorsed by current EU Water Framework Directives.[27,28] Chlorotriazine herbicides and primary monodealkylated metabolites (deisopropylatrazine and deethylatrazine) of recognized acute and chronic toxicity to biota[29] were selected as model compounds for evaluation of the potential applicability of the automatic bidimensional-μSPE methodology with in-line disposable beads for analysis of untreated complex environmental samples (e.g, ground waters from domestic rural wells and soil extracts).

Sequential injection (SI) analysis based on using programmable, bidirectional, discontinuous flow as precisely computer controlled and coordinated by resorting to syringe pump(s)[5,20,30] has been herein selected as a flow approach for precise metering and automatic handling of given volumes of solutions, organic washing solvents, air, and bead suspensions at the low microliter level, for processing large sample volumes for enrichment and matrix cleanup purposes, and as an appropriate front-end to LC. Further, the versatility of the flow setup devised has been exploited for postcolumn processing of the BI eluate prior to online injection into LC to prevent band broadening of the most polar analytes.

EXPERIMENTAL SECTION

Chemicals and Solutions. Chlorotriazine herbicides, namely, atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine), simazine (6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine) and propazine (6-chloro-N^2,N^4-diisopropyl-1,3,5-triazine-2,4-diamine), and their primary monodealkylated metabolite

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products, namely, deisopropylatrazine (6-chloro-*N*-ethyl-1,3,5-triazine-2,4-diamine; DIA) and deethylatrazine (6-chloro-*N*-(1-methylethyl)-1,3,5-triazine-2,4-diamine; DEA), were obtained from Sigma-Aldrich (Steinheim, Germany). Prometon (N^2,N^4-diisopropy-6-methoxy-1,3,5-triazine-2,4-diamine) was selected as internal standard for the chromatographic assays. The individual stock solutions of 20 μg mL^{-1} of DEA, atrazine, simazine, and propazine were prepared in methanol by diluting 2 mL of 100 μg mL^{-1} each to a final volume of 10 mL. The stock solutions of 10 000 μg mL^{-1} of DIA and prometon were prepared by dissolving 250 mg of each individual compound in pure methanol. All standards were stored in the darkness at 4 °C and stepwise diluted to the desired concentration for preparation of working solutions. The standard mixtures of triazines for manual injection into LC were prepared in 20% (v/v) acetonitrile/water to prevent band broadening effects. Ultrapure water was obtained from a Milli-Q water generator (Synthesis A10, Milllipore, Billerica, MA). HPLC-grade methanol and acetonitrile were supplied by Merck (Darmstadt, Germany) and toluene CHROMASOLV for HPLC by Sigma-Aldrich. Toluene was further purified by percolation through an Oasis HLB cartridge (200 mg, 6 mL) to remove potential impurities that might be further retained onto the packed sorbent material by nonspecific reversed-phase interactions and interfere with the chromatographic separation.

Sorbent bead materials used for cleaning up of raw samples and preconcentrative extraction of targeted chlorotriazines and metabolites thereof involved the class-specific, terbuthylazine-imprinted polymer from MIP technologies AB (Lund, Sweden) and supplied by Supelco (Bellefonte, PA) as SupelMIP SPE (SPE Triazine 10, Steinheim, Germany), *N*-vinylpyrrolidone−divinylbenzene copolymer (Oasis HLB, 30 μm, Waters, Mildford, MA), and spherically shaped octadecyl chemically modified silica (Upti Clean C18, 50 μm, Interchim, Montluçon, France).

Liquid Chromatograph. Liquid chromatographic assays were performed by resorting to HPLC 1100 system from Agilent Technologies (Palo Alto, CA) consisting of a vacuum degasser, a quaternary pump, a thermostat, and a UV/vis diode-array detector. Manual injections were conducted using a Rheodyne high-pressure six-port rotary valve (series 7725i) equipped with a 350 μL stainless steel loop (0.5 mm i.d. × 178.3 cm long). This valve was also exploited as the interface between the flow system and the analytical column. The target chlorocompounds and principal metabolite products thereof were separated and determined on a series of C18 reversed-phase guard column (Kromosil 100 C18, 5 μm, 15 × 4.6 mm, Scharlab, Barcelona, Spain) and analytical packed-bed column of identical chemical composition (Kromosil 100 C18, 3.5 μm, 150 × 4.6 mm, Scharlab) by a linear gradient elution from 20:80 to 70:30 (v/v) acetonitrile/water in 20 min at a flow rate 1.0 mL min^{-1}. Column temperature was controlled at 40 °C throughout the assays. Eluted chlorotriazines were monitored simultaneously at 220 and 225 nm and quantified by internal calibration based on prometon as internal standard using peak area measurement. Running of the LC gradient sequence, recording of chromatogram peaks, and data processing were performed automatically by a PC operated under the Chem Station Rev.10.01 software (Agilent).

Flow Setup. A multisyringe piston pump with programmable speed (MicroBu 2030, Crison Instruments, Alella, Barcelona, Spain) was used as a liquid driver for automation of the μSPE operations. It was equipped with two high-precision bidirectional syringes (Hamilton, Switzerland), labeled as S1 and S2 in Figure 1, with a capacity of 5.0 mL each and connected in block to a 40 000-step motor. S1 and S2 both contained Milli-Q water and were used for fluid delivery and postcolumn remixing of the organic eluate with Milli-Q water prior to LC separation, respectively. A three-way solenoid valve (N-Research, Caldwell, NJ) was placed at the head of each syringe enabling automatic connection with either the liquid reservoirs (OFF) or the flow network (ON). The multisyringe module was coupled to an eight-port multiposition selection valve operating as sample-processing unit (MPV, Valco Instruments, Houston, TX). The selection valve encompasses an ancillary central port and a communication channel (CC) that can be programmed to address each of the peripheral ports. The selection valve was connected via a 3.0 mL holding coil (HC, 1.5 mm i.d PTFE tubing) to S2 for microfluidic handling of the various components of the μSPE procedure (viz., sample, eluent, bead suspensions, and air). Bead containers made of 1 mL pipet tips were mounted vertically on ports 5 and 6 of the MPV using PEEK connectors for MIP and Oasis HLB, respectively. Bead suspensions of 1:4 (w/v) were prepared in pure methanol and 60% (v/v) methanol/water for Oasis HLB and MIP sorbents, respectively. The remaining ports were used for sequential aspiration of sample (port 3), air (port 4), methanol (port 7), and toluene (port 8) or as waste (port 2). The selection valve was connected via a 200 μL transfer line (1.5 mm i.d. PTFE) to a six-port rotary injection valve (IV, Valco) furnished with a cylindrical column (2 mm i.d., internal volume 25 μL) from transparent Kel-F fluoroplastic working as in-line container for capturing of the beads for renewable multimodal μSPE columns. The outlet of the microcolumn was equipped with a 10-μm polyethylene frit (Mo Bi Tec, Göttingen, Germany) for efficient trapping of the sorbent surfaces. The overall IV ports and internal channels were drilled to 1.5 mm i.d. and 2 mm width, respectively, for fostering the reproducible packing and removal of the sorbent column by programmable flow. Otherwise, undue flow impedance was eventually observed. A miniaturized Laboport diaphragm pump (65 W, KNF lab, Freiburg, Germany) was attached to a peripheral port of IV (port 5) for sorbent drying within the μSPE procedure. The IV outlet (port 2) and the delivery line from S1 merged at a Kel-F T-piece, which was connected with the high-pressure IV of LC via a 70 μL transfer PTFE line (0.8 mm i.d.). A schematic illustration of the hyphenated analytical setup for in-line selective preconcentration and chromatographic separation of targeted triazines is depicted in Figure 1. The operational procedures of the automatic μSPE procedure were computer controlled by the software package AutoAnalysis 5.0 (Sciware, Palma de Mallorca, Spain) based on dynamic link libraries (DLLs).

Sample Preparation and Characterization. *Soil Samples and Extraction Procedure.* Two different surface soils from agriculture sites in Mallorca (coded soil 1 and soil 2) were selected to investigate the reliability of the proposed flow assembly. Prior to chemical analysis, soils were oven-dried at 45 °C until constant weight and 2-mm sieved. Soil pH was measured in 0.01 mol L^{-1} $CaCl_2$ at a soil to solution ratio of 1:5 (w:v) after 2 h of

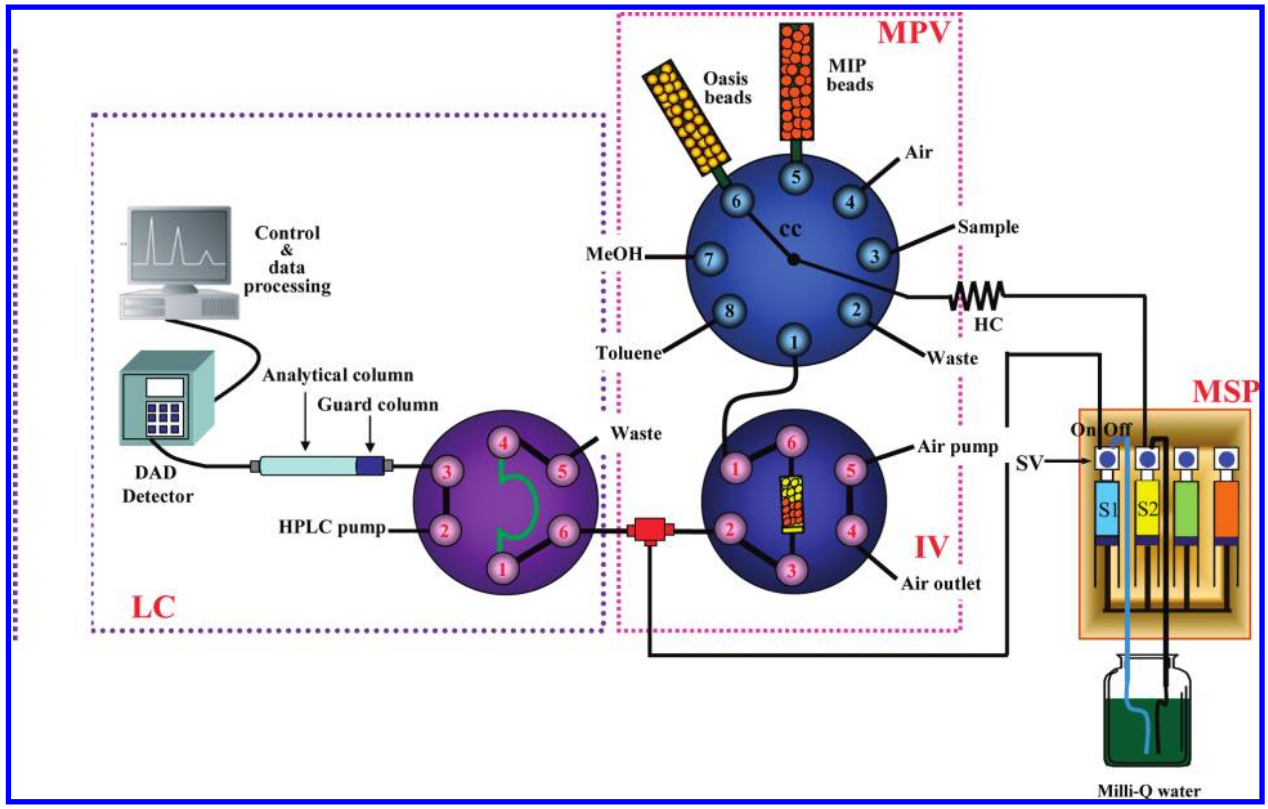

Figure 1. Schematic diagram of the hybrid flow system for automatic multimodal μSPE of trace level concentrations of chlorotriazines utilizing renewable surfaces as a front end to liquid chromatography. MSP, multisyringe pump; S, syringe pump; SV, solenoid valve; IV, injection valve; MPV, multiposition selection valve; HC, holding coil, CC, communication channel; DAD, diode-array detector.

equilibration using a combined pH electrode as endorsed by ISO 10390.[31] pH values of 7.52 ± 0.03 and 7.64 ± 0.07 were obtained for soil 1 and 2, respectively. Total organic carbon contents (TOC) of 8.55% and 8.80% for soil 1 and 2, respectively, were determined by dry combustion at 900 °C following release of carbonates with a few drops of 20% (v/v) HCl solution. Particle size distribution of the fraction <2 mm for determination of soil texture was performed with the Bouyoucos hydrometer method (ASTM type 152H).[32] Soil 1 consisting of 51.1% sand (0.05−2.0 mm), 34.5% silt (2−50 μm), and 14.4% clay (<2 μm) and soil 2 of 53.0% sand, 35.4% silt, and 11.6% clay, respectively, were classified as loam and sandy loam soil, respectively.

Surrogate contaminated soils were prepared by doping 80 g of sample with chlorotriazines and their principal metabolite products at two different concentration levels (20 and 50 ng g^{-1}) using a standard mixture containing 100 ng mL^{-1} each in acetone. Prometon was used as internal standard at the 50 ng g^{-1} level. A metered volume of acetone was added until the solvent completely covered the soil particles, whereupon the samples were gently stirred to ensure homogenization. Doped soils were air-dried at room temperature overnight and extracted thereafter. To this end, 5 g of soil (3 replicates each) was extracted in 40 mL of a dichloromethane/methanol (9:1) mixture as recommended by Chapuis et al.[33] with the aid of ultrasonic energy (150 W, 50 Hz, P-Selecta Asincro, Spain) for 15 min. The extract was filtered through 0.45-μm cellulose acetate membrane and the filtrate was evaporated gently to dryness by a nitrogen stream. The solid residue was dissolved in 150 μL of pure methanol with ultrasonic assistance for 3 min, to which 3 mL of Milli-Q water were added. The methanolic solution was filtered and made up to a final volume of 5 mL with Milli-Q water prior to analysis by the automatic SI−BI−HPLC assembly.

Water Samples. Drinking tap water (Palma de Mallorca), domestic well water (Valldemossa, Mallorca), and potentially contaminated creek water from a rural site (Muro, Mallorca) were analyzed for chlorotriazine content. Water samples were vacuum filtered through 0.45-μm cellulose esters filters (Millipore) previously rinsed with pure methanol and analyzed without pH adjustment.[34] For recovery tests, the overall water samples were spiked at the 0.5 and 2.0 ng mL^{-1} levels with the target herbicides and metabolites thereof using prometon as the internal standard at the 5 ng mL^{-1} level.

Analytical Procedure. The complete operational sequence of the hyphenated analytical setup for automatic preconcentration, separation, and determination of target triazine herbicides in untreated waters and crude soil extracts encompassed the injection of the multimodal beads and packing of the tandem μSPE column, conditioning of the column, sample loading, rinsing or solvent switch, analyte elution, injection of eluate into LC, and

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(32) Dietrich, H. *SOP METH004.00: Standard operating procedure for determining soil particle size using the hydrometer method*; Environmental Monitoring Branch, California Department of Pesticide Regulation: Sacramento, CA, 2005.

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(34) Ferrer, I.; Hennion, M. C.; Barceló, D. *Anal. Chem.* **1997**, *69*, 4508–4514.

discarding beads. The operational details of the overall analytical procedure are detailed in the following.

Sorbent Packing and Equilibration. First, S2 was set to aspirate consecutively 100 μL of air, 100 μL of MeOH, and 30 μL of MIP suspension in 60% (v/v) methanol/water from the external reservoirs into HC, whereupon the MIP beads were delivered by flow reversal to IV, captured in the external microcolumn, and rinsed by methanol and ancillary 600 μL of carrier (Milli-Q water). An identical procedure was programmed for aspiration and trapping of 10 μL of Oasis HLB suspension to generate the multimodal μSPE tandem column. Flow rates for aspiration of bead suspensions and solutions into HC were affixed to 0.3 and 1.5 mL min^{-1}, respectively. Both sorbent materials were packed into the microcolumn container at 1.0 mL min^{-1}. Beads were further conditioned by consecutive delivery of 500 μL of MeOH and 500 μL of carrier.

Sample Loading. S2 was programmed to aspirate 100 μL of air and 2000 μL of water sample consecutively into HC for further perfusion through the renewable tandem column at 1.0 mL min^{-1}. These operational steps were 5-fold repeated in order to handle a total sample volume of 10 mL. The surplus of air in HC was delivered to waste to release the system pressure. Sorbent cleanup was effected by loading 800 μL of carrier solution through the packed microcolumn following sample percolation. For soil extracts, the sample volume perfused was affixed to 1000 μL.

Solvent Switch for Soil Extracts. Further cleanup with toluene was conducted for soil extracts to promote selective interactions of target species with the imprint sites within the MIP. To this end, compressed air was pumped for 10 min through the packed microcolumn once the IV was activated to the inject position in order to strip out the remaining water from the sorbent materials, which would otherwise give rise to partial losses of analytes in the washing step. At the same time, the line communicating this IV with MPV was filled with air. Thereafter, IV was switched to the load position and S2 was programmed to perfuse the analyte-containing tandem column with an air-segmented plug of toluene (800 μL) at 0.5 mL min^{-1}. Prior to elute the sorbed species, the multimodal μSPE column should be again dried with compressed air for 10 min to remove traces of toluene leftover within the column dead volume. The IV loop of LC was simultaneously rinsed by a 1000 μL MeOH plug at 1.5 mL min^{-1}.

Analyte Elution. Retrieval of preconcentrated triazine residues was accomplished by resorting to a 170 μL MeOH segment followed by an air plug (both delivered at 5 μL s^{-1}) to prevent dispersion of the eluate within the carrier solution. The eluate plug was merged downstream with an identical volume of water provided by S1 at 0.5 mL min^{-1} prior to entering into the injection loop of the high pressure IV, whereupon the valve was switched to the injection position, and thus, the LC gradient protocol was initiated. Hence, the LC separation was synchronized with the SI−BI procedure, whereby a given volume of aqueous sample or soil extract was analyzed while the ensuing one was being processed in the hybrid flow system. The automated operational sequence for a single sample lasted 30 min for waters and 40 min for soil extracts, thus matching the time frame of the chromatographic run and re-equilibration of the analytical column to the initial conditions, which amounted to 30 min.

Bead Discarding. Renewal of the trapped tandem column involved a preliminary step of sorbent moistening with 800 μL of MeOH at 1.5 mL min^{-1}, whereupon the beads were drawn into HC by flow-reversal at 1.0 mL min^{-1} and then dispensed into waste through port 2 by a carrier stream at 3.0 mL min^{-1}. Hence, the hybrid flow system is ready to initiate a new analysis cycle with a fresh portion of beads, thus overcoming the potential irreversible sorption of interfering matrix ingredients and sample cross-contamination as well between consecutive runs.

RESULTS AND DISCUSSION

Selection of Sorbent Material. Preliminary tests in a mesofluidic lab-on-a-valve platform[6,20,35,36] were conducted to ascertain the sorptive preconcentration capabilities of a series of bead materials with different functionalities for uptake of the overall triazine residues along with the more polar dealkylated metabolites in a μSPE format. Three different reversed-phase sorbent materials, namely, water-compatible MIP, copolymeric Oasis HLB, and Upti-Clean C18 silica beads, were investigated in this work as single-use solid extractants in the flow network. The latter two sorptive materials are spherically shaped and uniform in size distribution, thus fostering their reproducible in-valve manipulation and usage in a renewable mode. Despite the lump-type morphology of the commercially available MIP, automatic handling via programmable flow within the flow conduits was proven feasible by appropriate selection of the dispersion medium [in our case 60% (v/v) MeOH/H_2O] to retard bead settlement into the central processing valve unit.

Sorption capabilities of individual packed columns of Oasis HLB (3.0 ± 0.3 mg), Upti Clean C18 (7.0 ± 0.8 mg), and triazine-MIPs (3.2 ± 0.5 mg) were investigated for the enrichment of 1 mL of mix standard of chlorotriazines and chlorinated degradates thereof containing 100 ng mL^{-1} each. Elution was effected with 100 μL of pure MeOH. Oasis HLB was proven to be the most suitable sorbent material in a miniaturized SPE format for the uptake of both parent compounds and monodealkylated metabolites (see Figure 2). This is a consequence of the mixed-mode sorptive behavior of the copolymeric material bearing a balanced ratio of hydrophobic and hydrophilic moieties. On the other hand, the retention efficiency of less hydrophobic DIA and DEA onto the spherical C18 beads dropped by 30−50% as compared with the copolymeric Oasis HLB sorbent (see Figure 2). This also holds true for the MIP material under reversed-phase chemical interactions. This denotes the limited binding capacity of noncovalent MIP for uptake of moderately polar triazines in water matrices via nonspecific sorption onto the residual monomers at the outer surface of the polymer. Despite the miniature dimensions of the renewable in-line MIP reactor herein, this observation is in good agreement with earlier findings in dynamic MISPE using packed-bed permanent columns with >30-fold increased amount of sorbent.[25,37]

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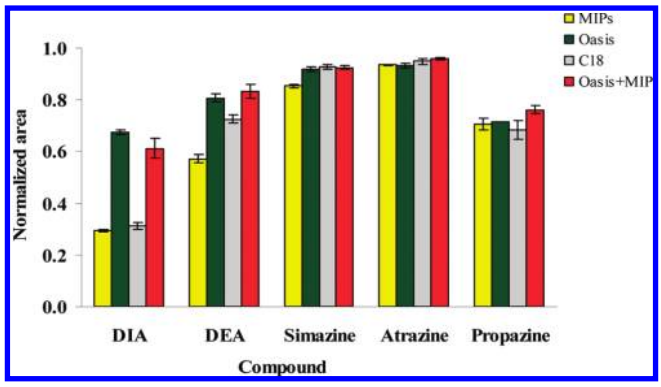

Figure 2. Effect of the chemical nature of the sorbents in a single-μSPE or tandem-μSPE column fashion in the uptake of chlorotriazines and dealkylated metabolites thereof from 1 mL of mixed standard at the 100 ng mL^{-1} level.

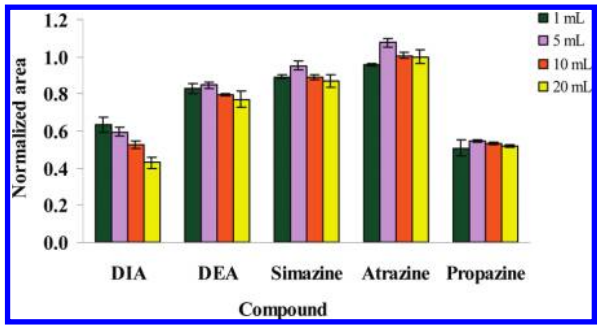

Figure 3. Investigation of breakthrough volumes for the μSPE−BI tandem column in the preconcentration of a given amount of chlorotriazines (100 ng) in 1, 5, 10, and 20 mL of 100, 20, 10, and 5 ng mL^{-1}, respectively. Analytes were eluted by 170 μL of pure methanol at 0.5 mL min^{-1}.

To tackle the aforementioned drawbacks that impede the processing of large volumes of untreated water samples onto MIP reactors, a flow-through multimodal renewable-bead sorptive procedure encompassing a prior uptake and enrichment of species onto *N*-vinylpyrrolidone−divinylbenzene copolymer (Oasis HLB) followed by further cleanup on MIP is herein proposed for preconcentration and determination of triazine residues at environmentally relevant levels. An in-loop microcolumn (in lieu of LOV) arrangement (see Figure 1) was devised to admit larger amounts of sorbents (2.7 ± 0.3 mg of Oasis HLB plus 7.0 ± 1.0 mg of MIP) and foster the efficient drying of beads prior to elution. The idea behind this configuration was to ensure the selective and repeatable sorptive preconcentation of target species when handling complex matrices. The improved recoveries for the less hydrophobic dealkylated metabolites compared to MIP alone (see Figure 2) demonstrated the suitability of this bidimensional μSPE procedure for processing samples containing chlorotriazines with a broad spectrum of polarities.

Investigation of the maximum enrichment factors attainable with the tandem column arrangement was performed in terms of breakthrough volumes. To this end, a given amount of target chlorotriazines (100 ng each) in a mixed standard was loaded onto the column in increasing sample volumes, as shown in Figure 3. No breakthrough was observed for any of the target species up to 20 mL, excepting for DIA, for which significant pre-elution was observed at volumes >10 mL (as compared to 1 mL). In fact, absolute recoveries of merely 51% for DIA have been earlier reported in online MISPE procedures when loading water volumes as low as 1.5 mL.[38] For appropriate preconcentration of the most polar metabolite residue and parent compounds as well, sample percolation volumes were affixed to ≤10 mL.

The sample loading and elution flow rates for the μSPE procedure were affixed to 0.5 and 0.3 mL min^{-1}, respectively, to ensure quantitative uptake of the overall target species including the more polar chlorinated degradates onto the miniaturized sorptive columns and efficient retrieval in a minimum volume of eluent for subsequent online injection into LC without pressure drop within the analytical sequence.

Online Coupling of Bead-Injection Tandem Column to LC. A survey of the literature revealed that online hyphenation of class-specific MIP sorbents with chromatographic separations is not a common practice.[10] This is most likely a consequence of the band-broadening of moderately polar species whenever the optimum hydroalcoholic MIP eluent with high methanol or ethanol content is delivered to the LC.[38,39] In our case, DIA and DEA could not be accurately quantified whenever volumes >50 μL in pure methanol were directly injected into LC because of undue peak dispersion and eventual coelution in the void volume.

To overcome the lack of compatibility of the eluent medium with the isocratic or gradient LC separation, a plethora of authors exploited online column-switching methods[7,40] involving the elution of the sorbed species with the mobile phase itself. This approach might, however, be inappropriate for attaining high enrichment factors, because of the incomplete elution of hydrophobic species or excessive spreading of the elution band. In-line heart-cut elution schemes[41] encompassing the injection of a small segment of solvent with increased elution strength into LC do not render improved sensitivity because of the partial loss of the preconcentration capabilities gained during analyte sorption.

In this work, a SI-based programmable elution mode was selected aimed at the introduction of the overall solvent volume of optimum elution strength into the LC. One of the cornerstones of flow-based systems is the controllable sample/reagent dispersion, which was initially exploited for in-tube dilution of the eluate band into the carrier stream toward the LC and within the injection loop itself. By this means, no appreciable band broadening for either triazine metabolite was observed for 150 μL of 90% (v/v) MeOH/H_2O or 120 μL of 100% MeOH utilizing the SI manifold described previously. Quantitative elution of the most hydrophobic parent compounds was, however, not accomplished. In fact, the higher the volume of pure methanolic eluent percolated through the tandem column, the better were the absolute recoveries of the target species up to 170 μL eluent, which was chosen for the remainder of the work.

To ensure quantitative stripping out of triazines from the multimodal-μSPE column and efficient LC band-focusing, the SI manifold was hyphenated with a multisyringe flow setup for appropriate in-line dilution. To this end, the 170 μL methanolic plug was merged downstream with a water-stream provided concurrently by one of the liquid drivers of the multisyringe device

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Table 1. Analytical Performance of the SI−BI Setup Hyphenated to LC for Determination of Trace Level Concentrations of Chlorotriazines at Varied Sample Volumes

procedure	compound	retention time (min)	normalized regression equation	correlation coefficient (r)	linear range (ng mL^{-1})	enrichment factor	absolute recovery (%)	RSD (%)	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
10 mL sample loading[a]	DIA	4.6	$y = 0.1830x - 0.0282$	0.9993	0.12−10	46	79	5.5	0.04	0.12
	DEA	6.9	$y = 0.2370x - 0.0232$	0.9997	0.1−10	49	83	2.1	0.03	0.1
	simazine	10.9	$y = 0.2378x - 0.0125$	0.9990	0.1−10	48	81	1.4	0.03	0.1
	atrazine	14.1	$y = 0.2332x - 0.0093$	0.9994	0.1−10	46	79	1.6	0.02	0.07
	propazine	17.1	$y = 0.1932x + 0.0060$	0.9998	0.1−10	47	80	3.5	0.03	0.1
1 mL sample loading[b]	DIA	4.6	$y = 0.0187x - 0.0016$	0.9984	5−100	4.0	68	6.0	0.5	1.7
	DEA	6.9	$y = 0.0249x + 0.0037$	0.9999	5−100	3.8	64	5.1	0.4	1.3
	simazine	10.9	$y = 0.0243x + 0.0288$	0.9998	5−100	3.1	52	4.3	0.5	1.5
	atrazine	14.1	$y = 0.0246x + 0.0395$	0.9995	5−100	3.0	51	5.0	0.3	1.1
	propazine	17.1	$y = 0.0210x + 0.0175$	0.9999	5−100	2.8	47	4.7	0.5	1.7

[a] Intended for water assays. [b] Intended for soil extract assays. The analytical method comprises in-line cleanup with toluene. Analytical wavelength: 220 nm. LOD and LOQ were calculated as S/N = 3 and 10 on the basis of the analytical signals at the 5 ng mL^{-1} level. Calibration standards: (a) 0.1, 0.5, 1.0, 2.0, 5.0, and 10 ng mL^{-1}; (b) 5.0, 10, 20, 50, and 100 ng mL^{-1}.

(S1) in a 1:1 water/eluent ratio (see Figure 1), thereby rendering a final eluate plug composition of 50% (v/v) MeOH/water, which satisfied the requirements for the LC separation.

Online coupling of noncovalent MIP with LC might be also troublesome as a result of remains of organic solvent from sorbent cleanup within the packed reactor, which might deteriorate further LC separation of target species. Method development experiments should thus include the exploration of appropriate washing solvents and in-line cleanup procedures as described below.

Analytical Performance. The beauty of the hyphenated hybrid flow system furnished with the tandem BI column lies in the fact that both the selectivity and sensitivity of analytical methods might be tuned at will attending the analysis needs. Detectability of triazine residues can be greatly enhanced by loading large sample volumes onto *N*-vinylpyrrolidone−divinylbenzene, yet analytes are retained primary by hydrophobic interactions and sorption is thus fairly nonspecific in nature. Further cleanup for removal of concomitantly adsorbed interfering substances might be accomplished via solvent switch to disrupt the hydrophobic interactions and in-line retention of chlorotriazines within the complementary imprinted sites of the bottom MIP. In order to change the retention conditions of target species to the selective normal-phase mode, a weakly polar and aprotic solvent is needed. Dichlomethane or toluene are the solvents of choice in noncovalent MIP-based triazine assays.[25,37,38,42] The former was proven inappropriate in our configuration because residual traces of solvent leftover after the washing step overlapped severely with simazine and interfere with other analyte peaks as well. On the other hand, possible remains of toluene after 10 min of drying as detailed previously did not interfere with either of the target peaks. Toluene was therefore selected for promoting hydrophilic, hydrogen-bonding-type interactions of chlorotriazines and metabolites thereof with MIP sites.

In order to assess the efficiency of the μMISPE column for selective uptake of triazines against chlorinated herbicides with similar chemical structure, polarity, and molecular size, diuron (*N*′-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea) was selected as a model compound for cross-reactivity studies. A mixed standard solution of target chlorotriazines at the 30 ng mL^{-1} each was doped with the interfering species at the same concentration level. Following loading of 1 mL standard onto the multimodal microcolumn, increasing volumes of toluene ranging from 0 to 1000 μL were percolated through the tandem microcolumn to foster the selective redistribution of the analytes from the Oasis HLB top sorbent to the imprinted sites of the bottom MIP. Experimental results revealed that the higher the volume of washing solvent, the better was the removal of diuron interference, yet concomitantly the most significant were the losses of the more hydrophobic triazines, which might jeopardize the sensitivity requirements of the assays. These results are in good agreement with earlier findings when handling noncovalent MIP in a batchwise MISPE mode.[18] By employing a rinsing volume of 800 μL toluene, diuron interference (peak area) was removed >80% and propazine and simazine were washed off ≤10%. Quantification of the parent structural analogues was accurately performed in the presence of diuron with deviations <10%.

Under the optimized chemical and physical variables detailed in the foregoing sections, the figures of merit of the hybrid flow system are summarized in Table 1, including retention times, calibration graphs, dynamic linear ranges, enrichment factors, absolute recoveries, repeatability, reproducibility, and sensitivity for further determination of the suite of herbicides in either untreated water samples or crude extracts. In the former, 10-mL samples doped with 5 ng mL^{-1} internal standard (prometon) were perfused through the packed beads and analyzed without solvent switch (see the section on real sample analysis). In the latter, 1-mL samples spiked with 50 ng mL^{-1} prometon were processed in the flow setup and analyzed by LC following bidimensional BI-μSPE with toluene washing.

For quantification of the parent triazines and dealkylated metabolites, five-level calibration plots were exploited in the entire set of assays with determination coefficients >0.9984. Concentration ranges spanned over 2 orders of magnitude, that is, 0.1−10 or 5−100 ng mL^{-1}, in accordance with the standard/sample volume percolated. Absolute recovery percentages were calculated as the ratio of the peak areas in the online SI−LC methods and those from 100 μL direct chromatographic injection of an equivalent mass in a medium matching the initial chemical composition of the LC gradient elution. Enrichment

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Table 2. Concentrations and Recoveries of Target Chlorotriazines and Metabolites in Untreated Environmental Waters[a]

	tap water		ground water		creek water	
compound	2.0 ng mL^{-1} spiked (recovery, %)	0.5 ng mL^{-1} spiked (recovery, %)	2.0 ng mL^{-1} spiked (recovery, %)	0.5 ng mL^{-1} spiked (recovery, %)	2.0 ng mL^{-1} spiked (recovery, %)	0.5 ng mL^{-1} spiked (recovery, %)
DIA	1.95 ± 0.07 (98)	0.54 ± 0.02 (108)	2.07 ± 0.07 (104)	0.59 ± 0.02 (119)	2.05 ± 0.09 (103)	0.56 ± 0.01 (112)
DEA	1.89 ± 0.03 (95)	0.47 ± 0.03 (94)	2.06 ± 0.02 (103)	0.52 ± 0.05 (104)	1.97 ± 0.04 (98)	0.44 ± 0.01 (88)
simazine	1.92 ± 0.04 (96)	0.46 ± 0.07 (92)	2.00 ± 0.04 (100)	0.53 ± 0.01 (106)	1.88 ± 0.03 (94)	0.46 ± 0.02 (92)
atrazine	1.96 ± 0.04 (98)	0.57 ± 0.05 (114)	1.96 ± 0.03 (98)	0.51 ± 0.01 (101)	1.93 ± 0.02 (96)	0.47 ± 0.03 (94)
propazine	1.75 ± 0.02 (88)	0.48 ± 0.06 (96)	1.91 ± 0.01 (96)	0.50 ± 0.01 (100)	1.91 ± 0.04 (96)	0.43 ± 0.01 (86)

[a] Sample volume: 10 mL.

Table 3. Concentrations and Recoveries of Target Chlorotriazines and Metabolites in Crude Extracts of Agriculture Soils[a]

	soil 1		soil 2	
compound	50 ng g^{-1} spiked (recovery, %)	20 ng g^{-1} spiked (recovery, %)	50 ng g^{-1} spiked (recovery, %)	20 ng g^{-1} spiked (recovery, %)
DIA	55.7 ± 4.8 (111)	22.8 ± 5.5 (114)	55.8 ± 2.1 (112)	24.0 ± 1.4 (120)
DEA	46.3 ± 3.4 (93)	16.8 ± 2.8 (84)	52.7 ± 1.0 (105)	22.3 ± 1.6 (112)
simazine	49.9 ± 2.4 (100)	20.6 ± 3.5 (103)	50.9 ± 0.6 (102)	23.9 ± 4.7 (120)
atrazine	44.7 ± 1.4 (89)	18.2 ± 3.3 (91)	46.8 ± 0.4 (94)	20.1 ± 1.9 (100)
propazine	45.0 ± 1.0 (90)	21.2 ± 2.8 (106)	43.3 ± 0.5 (87)	21.1 ± 0.4 (106)

[a] Sample volume: 1 mL.

factors were calculated as the ratio of the linear range sensitivity of the proposed SI−preconcentration procedures and that obtained by direct injection of 170 μL standard solutions into LC.

Method repeatability was expressed as the precision obtained from six consecutive measurements of a 10 mL mixed standard solution at the 2 ng mL^{-1} level using a permanent multimodal sorbent column. Relative standard deviations ranging from 1.4% to 5.5% (see Table 1) were better than repeatability values previously reported in batchwise MISPE procedures for determination of chlorotriazines where RSDs varied from 9 to 18%[25] or 4 to 8%[42] and batchwise MISPME where RSDs of 5.7%−10.6%[43] and 4−10%[44] were reported. The overall procedure reproducibility was expressed as the RSD of six consecutive analysis of 1 mL mixed standard at the 20 ng mL^{-1} level exploiting renewable surfaces. The automatic SI−tandem column BI method rendered improved RSDs for triazines (4.3−6.0%) as compared to manual MIP-based extraction protocols with reproducibility values of 7−15%[44] or 5−10%.[42]

The LODs and LOQs, calculated at a peak-to-peak signal-to-noise ratio (S/N) of 3 and 10, respectively,[45,46] for analysis of 10 mL-spiked water at the 0.5 ng mL^{-1} level ranged from 0.02−0.04 to 0.07−0.12 ng mL^{-1}, respectively (see Table 1). Therefore, the automatic μSPE procedure fully meets the requirements endorsed by the current EU Water Framework Directives[27,28] for determination of triazines at environmentally relevant levels, where the maximum allowed concentrations of atrazine and simazine in surface waters are set to 2.0 and 4.0 ng mL^{-1}, respectively, and <0.1 ng mL^{-1} in tap waters. In fact, LODs are even better than those earlier reported in off-line MISPE of 100 mL water samples prior to LC separation (0.05−0.2 ng mL^{-1}).[25] Compared to alternative preconcentration/separation procedures for triazine assays in aqueous media, e.g, in-line MISPE−capillary electrophoresis with LODs within 0.2−0.6 μg mL^{-1},[47] electrochemical sensing with LOD of 0.2 μg mL^{-1},[48] or MISPME coupled to GC/MS with LODs from 0.02 to 0.09 μg mL^{-1},[49] the proposed flow-through procedure features 3−4 orders of magnitude better LODs.

Applicability to the Analysis of Untreated Water Samples and Crude Extracts. To assess the reliability and ruggedness of the flow-based analytical method, real-life samples of variable matrix complexity and nature, that is, environmental waters and soil extracts, were processed with minimum prior sample treatment. Due to the lack of certified reference materials containing both triazines and dealkylated metabolites thereof, surface and ground waters and soils were doped with the overall analytes at environmentally relevant levels (see Tables 2 and 3).

Untreated Environmental Waters. Preliminary assays demonstrated that preconcentration and cleanup of tap, surface, and ground waters onto *N*-vinylpyrrolidone−divinylbenzene sufficed for the accurate determination of chlorotriazines at the concentration levels specified by EU directives for water bodies. Thus, 10 mL of untreated water samples was processed in the proposed SI assembly without solvent switch to ensure appropriate sensitivity. In addition, the in-valve μSPE column could be reused up to six injections without deterioration of the analytical performance. Relative recoveries of analytes in spiked water (tap, creek, and well) samples at the 0.5 and 2.0 ng mL^{-1} levels are summarized

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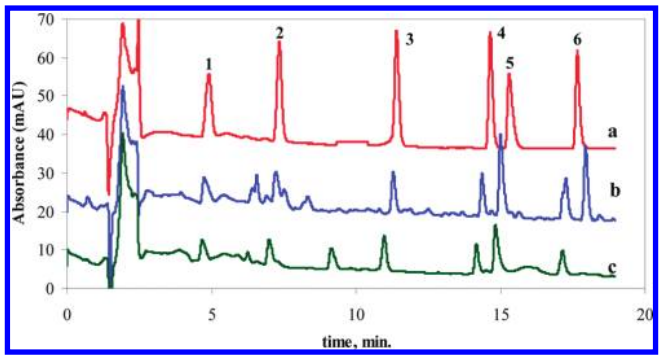

Figure 4. Chromatograms for the determination of chlorotriazines and dealkylated metabolites in (a) 100 μL of 1000 ng mL^{-1} of target compounds manually injected into LC, (b) the eluate of tandem-μSPE column following in-line processing of 1 mL of soil extract without solvent switch, and (c) the eluate of tandem-μSPE column following in-line processing of 1 mL of soil extract with further rinsing with 800 μL of toluene. Soil sample was spiked at the 20 ng g^{-1} level with 1 = deisopropylatrazine, 2 = deethylatrazine, 3 = simazine, 4 = atrazine, 6 = propazine. The internal standard (prometon, peak 5) was spiked at the 50 ng g^{-1} level. Analytical wavelength: 225 nm. Impurities of toluene eluted in part c between DEA and simazine. Shifts of more hydrophobic compounds in c part are a consequence of the remains of toluene in the system, which works as an organic HPLC modifier.

in Table 2. Satisfactory recoveries ranging from 88 to 104% at the 2.0 ng mL^{-1} level and from 86 to 119% at the 0.5 ng mL^{-1} level were encountered for the suite of analyzed samples. The relative recoveries of triazines herein reported are better than those earlier published for batchwise MISPE using large amounts of sorbents.[25,50]

Crude Soil Extracts. As a result of concomitantly extracted interfering components in the soil extracts, a further cleanup of the sample following uptake of the target species onto *N*-vinylpyrrolidone−divinylbenzene or surface moieties of MIP was proven necessary. Thus, molecular recognition of the triazines by the imprinted sites of the MIP material was here fostered via toluene washing. A significant decrease in the number of interfering species, good baseline separation, and accurate quantification of the overall triazines (particularly DEA and propazine in the assayed samples) were attained following solvent switch onto the bidimensional BI column (see chromatograms in Figure 4). The cleanup procedure was distinctly more efficient for removal of the more hydrophobic compounds. Bead materials were automatically renewed after processing each individual sample or replicate. In order to minimize the eventual interfering effects on the dealkylated metabolites, analytical wavelengths were recorded simultaneously at 220 and 225 nm for further data processing. Relative recoveries in the analysis of 1 mL of agriculture soils doped at the 20 and 50 ng g^{-1} levels ranged from 84 to 120% (see Table 3). Similar or even better recoveries than those early reported for soil extracts using SPE/SPME-based methods prior to chromatographic separations[42,43,51,52] were here obtained. Sorbent expenses for single-use tandem columns amounted to <1.9 Euros per assay of soil samples.

CONCLUSIONS

In this paper, the proof-of-concept of renewable MIP as a μSPE reactor in an SI setup prior to chromatographic separations has been demonstrated for processing of crude soil extracts and determination of organic contaminants. Due to the low concentration levels of triazines and the effect of interfering matrix ingredients, multimodal SPE involving two kinds of beads (reversed-phase copolymeric and MIP sorbents) was employed aimed at the concomitant enhancement of selectivity and retention efficiency of target compounds in the sorptive extraction procedure. In-line μSPE with merely *N*-vinylpyrrolidone−divinylbenzene is recommended for environmental water assays in order to simplify the system without compromising the selectivity. After appropriate scrutiny of the various parameters governing the performance of the system, the flow analyzer provides sufficient sensitivity and reliability for long-term assays at concentration levels of herbicides below those endorsed by the current legislations for human water consumption and surface waters.

As an appealing "front-end" to LC, the hybrid flow system herein proposed has proven suitable to not merely handle both aqueous and organic solutions in a single automatic setup encompassing in-line sample pretreatment but effectively solve LC band-broadening effects via in-line dilution of the SPE eluate.

Further research is to be focused on expanding the developed MIP-based flow-through analyzer to the preconcentration, purification, and determination of trace level concentrations of other priority xenobiotics and endogenous organic compounds in a vast number of matrices including environmental and biological samples and foodstuffs as well.

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CHAPTER 8

Flow-through dispersed carbon nanofiber-based microsolid-phase extraction coupled to liquid chromatography for automatic determination of trace levels of priority environmental pollutants

analytical chemistry

ARTICLE

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Flow-through Dispersed Carbon Nanofiber-Based Microsolid-Phase Extraction Coupled to Liquid Chromatography for Automatic Determination of Trace Levels of Priority Environmental Pollutants

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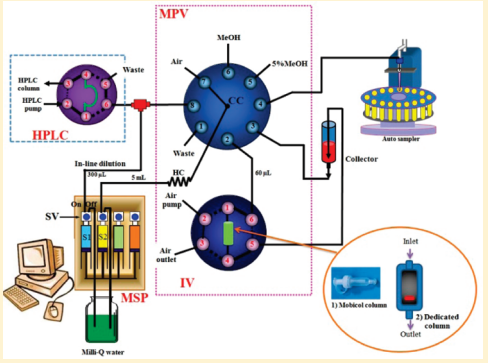
Supporting Information

ABSTRACT: Handling of carbon nanoparticles as sorptive materials in a flow-through packed-bed mode has been to date hampered by undue pressure drop and deteriorated retention efficiency because of nanoparticle bundling and entanglement. To surmount this limitation, a dedicated stirred-flow sorptive microchamber integrated in a fully automated sequential injection (SI) assembly is herein proposed for expedient handling and reuse of carbon nanoparticles in microsolid-phase extraction (μSPE) procedures. The assembled setup features automatic uptake, preconcentration, and retrieval of target organic species using dispersed nanoparticles as a front-end to liquid chromatographic (LC) assays. Chlorotriazine residues (atrazine, simazine, and propazine) and dealkylated metabolites thereof (deisopropyltriazine (DIA) and deethylatrazine (DEA)) were selected as model compounds because of their electron-poor aromatic structure and potentially strong $\pi-\pi$ interactions with electron-rich sorptive materials. The effect of several parameters on the analytical performance including the type and amount of nanoparticles (carbon nanofibers (CNFs), multiwalled carbon nanotubes (MWCNTs) and oxidized carbon nanotubes (MWCNT-COOH), the sample volume (breakthrough volume), the nature and volume of eluent, and the interface between the sample processing module and LC was explored in detail. Using dispersed CNFs at-line coupled to LC, absolute recovery percentages for 10 mL sample percolation were >94% for the overall herbicides with enrichment factors of ca. 20, limits of detection ($S/N = 3$) of 0.004−0.03 ng mL^{-1}, limits of quantification ($S/N = 10$) of 0.01−0.09 ng mL^{-1} and repeatability within the range 0.5−1.8%. The SI-CNF-LC hyphenated system was harnessed to the analysis of not merely untreated environmental waters at concentration levels below those endorsed by the current EU Water Framework Directives but to crude soil extracts for which CNF reuse with no loss of retention efficiency was proven feasible by resorting to appropriate automatic regeneration procedures and internal standardization.



Carbon nanoparticles encompassing carbon nanotubes (CNTs) or nanofibers (CNFs) (also termed cup-stacked nanotubes or nanowires) are rapidly evolving as sorptive materials and (pseudo)stationary phases in modern separation sciences[1−7] because of their unique physicochemical and mechanical properties and large chemically active surface area. The excellent adsorption capabilities of CNTs or CNFs can be attributed to the distortion of planar graphene sheets into a helical or cylindrical fashion. As a consequence, carbon nanoparticles readily experience fluctuating and induced dipole moments, which results in excellent van der Waals adhesion to organic species.[1,5,6] This effect along with their ability to establish noncovalent $\pi-\pi$ stacking interactions accounts for improved extraction capacity of either nonpolar or moderately polar organic compounds bearing aromatic moieties when compared to reversed-phase sorbent materials (e.g., octadecyl-chemically modified silica gel, polystyrene-divinylbenzene, and mixed-mode copolymers).[1,5,8]

In-house CNT-packed cartridges containing pristine or chemically functionalized sorbents have been harnessed to the preconcentration, cleanup, and determination of a vast number of xenobiotics or endogenous organic species in a batchwise manual mode.[1−3,5,6] Implementation of dedicated CNT-packed columns in flow-based systems as a permanent component of the manifold for automation and simplification of sample processing has been as of yet rather troublesome because of entanglement and bundling of the nanoparticles. As a result, progressive tighter packing and undue flow resistance has been

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observed in the course of sample loading and elution.[9,10] The use of minute amounts of sorbents (6−10 mg)[9,10] and large column aspect ratios to alleviate flow backpressure might however jeopardize the extraction efficiency of target species. In addition, the sorption capacity of CNTs in a packed column is far less than the nominal value because the effective surface area is reduced to a large extent as a result of nanoparticle agglomeration.

A great deal of attention has been recently devoted to the fabrication of carbon nanocomposites in a core−shell format using inorganic support materials to surmount the above shortcomings of CNTs in a flow-through sorptive mode.[1] Two recently reported procedures involved the isolation of individual vertically aligned nanotubes onto the surface of controlled pore glass by covalent immobilization[11] and the dynamic decoration of silica spheres with a thin layer of CNTs using a polyelectrolyte-assisted layer-by-layer surface assembly approach.[12] Both analytical procedures are however tedious and time-consuming and might lack appropriate between-batch reproducibility. Noncovalent wrapping of CNTs with polyelectrolytes should be regarded as a streamlined alternative,[13] yet the surface characteristics of CNTs are in this case altered, whereby reduced sorption efficiency might be expected toward hydrophobic species.

The immobilization of CNTs into the pore structure of polymeric membranes[14,15] might overcome sorbent aggregation. On the other hand, CNT-based sorptive disks are prone to early analyte breakthrough when used in a dynamic flow-through mode as a consequence of limited uptake capacity.[13] Thorough optimization of the sample loading flow rate is also needed to minimize the build-up of backpressure and ensure long-term optimum analytical performance with minimum operational maintenance.

In this work, a simple approach is proposed for automatic handling of carbon nanoparticles as μSPE materials in flowing streams with no need for nanocomposite fabrication, membrane decoration, or usage of supramolecular organized media. The method capitalizes on the design and exploitation of a flow-through stirred reactor for dispersion of the nanosized particles in a fully automated sequential injection (SI) setup. At-line coupling of the sorptive microchamber to liquid chromatography (LC) fosters both quantitative desorption of sorbed species and in-line processing of eluates without jeopardizing the ensuing reversed-phase chromatographic separation.

Chlorotriazines and dealkylated metabolites have been selected as model analytes for evaluation of the analytical performance of the SI-μSPE-LC hyphenated system. The assembled setup is aimed at preconcentration and determination of triazine residues with a broad spectrum of polarities in environmental waters at concentration levels well below the maximum allowed concentrations endorsed by the current European Water Framework Directive (2008/105/EC).[16] The potential application to crude soil extracts for triazine assays and automatic regeneration of the sorptive material by flow programming is investigated in detail. The vast majority of applications of CNTs for triazine residues lie in the field of analysis of aqueous environmental samples in a batchwise mode.[12,17−23] CNTs have been recently harnessed to SPE of more troublesome matrixes, such as crude soil extracts, yet exploiting single-use cartridges because of severe matrix effects.[23,24] As a result, no flow-through SPE method with reusable carbon nanoparticles for assays of soil extracts has been reported to date.

EXPERIMENTAL SECTION

Chemicals, Solutions, and Samples. Chlorotriazine herbicides, namely, atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine), simazine (6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine), and propazine (6-chloro-N^2,N^4-diisopropyl-1,3,5-triazine-2,4-diamine) and their primary monodealkylated metabolite products, viz., deisopropylatrazine (6-chloro-*N*-ethyl-1,3,5-triazine-2, 4-diamine; DIA) and deethylatrazine (6-chloro-*N*-(1-methylethyl)-1,3,5-triazine-2,4-diamine; DEA) were purchased from Sigma-Aldrich (Steinheim, Germany). Linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea (Riedel de Haën, Seelze, Germany)) was selected as internal standard for the extraction and chromatographic assays of herbicides in soils. The individual stock solutions of 20 μg mL^{-1} each of DEA, atrazine, simazine, and propazine were prepared in methanol by diluting 2 mL of 100 μg mL^{-1} each to a final volume of 10 mL. The stock solutions of 10 000 μg mL^{-1} of DIA and linuron were prepared by dissolving 250 mg of individual compounds in pure methanol. All standards were stored in the darkness at 4 °C and stepwise diluted to the desired concentration for preparation of working solutions. The standard mixtures of triazines for manual injection into LC were prepared in 20% (v/v) methanol/water to prevent band-broadening effects. Ultrapure water was obtained from a Milli-Q water generator (resistivity >18 MΩ cm; Millipore Synthesis A10, Billerica, MA). HPLC-grade methanol and acetonitrile were supplied by Merck (Darmstadt, Germany). Carbon nanofibers (diameter: 70−150 nm, length: >20 μm, surface area: 0.4 $cm^2 \mu g^{-1}$ and bulk density: 0.09 g mL^{-1}) from Electrovac AG (Klosterneuburg, Austria) were used as purchased for preconcentrative isolation of targeted chlorotriazines and for cleanup of raw environmental samples. Multiwalled carbon nanotubes (MWCNTs) and carboxylated multiwalled carbon nanotubes (MWCNT-COOH) with an average diameter of 10 nm and length of 1−2 μm (DropSens, Oviedo, Spain) were evaluated as sorptive nanomaterials as well.

Characterization of the environmental samples assayed and detail of the extraction procedure for agricultural soils are available in Supporting Information (text and Table S1).

Liquid Chromatography. Liquid chromatographic assays were performed by resorting to HPLC 1100 system from Agilent Technologies (Palo Alto, CA) consisting of a vacuum degasser, a quaternary pump, a thermostat, and a UV/vis diode-array detector. Manual injections were conducted using a Rheodyne high-pressure six-port rotary valve (series 7725i) equipped with a 350 μL stainless steel loop (0.5 mm i.d. × 178.3 cm long). This valve was also exploited as an online interface between the SI system and the analytical chromatographic column. The target chlorotriazines and principal metabolite products were separated on a series of octadecyl-chemically modified silica gel reversed-phase columns including the Chromguard precolumn (10 × 3 mm, Varian, Walnut Creek, CA) and the Nucleosil 100-5 C18 analytical column (250 × 4.6 mm, 5 μm, Varian), exploiting a linear gradient elution from 30:70 to 80:20 (v/v) acetonitrile/water in 20 min at a flow rate of 1.0 mL min^{-1}. Column temperature was kept at 40 °C throughout the assays. Chlorotriazines were all monitored at 220 nm and quantified by external calibration in water matrixes and linuron-based internal calibration in soil extracts. Running of the LC-gradient sequence,

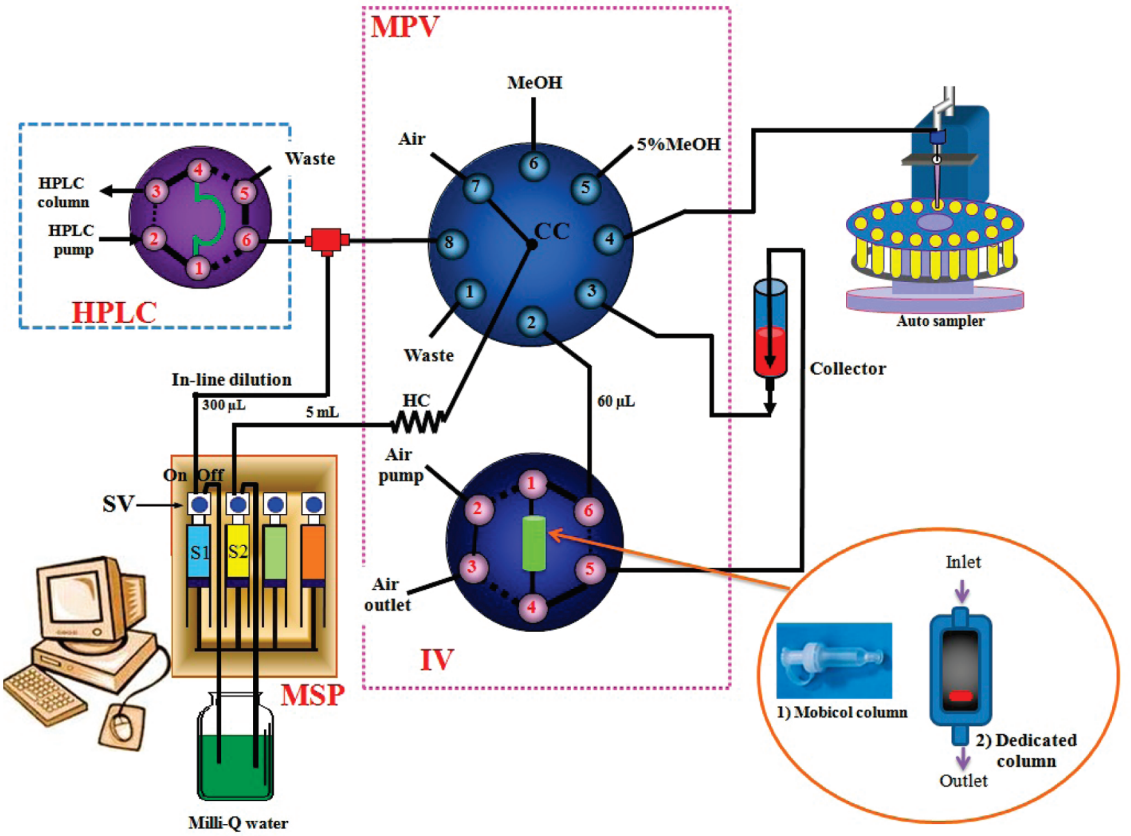

Figure 1. Schematic illustration of the SI-LC hyphenated setup for automated CNF-based microextraction and determination of trace level concentrations of triazine herbicides in environmental matrixes. MPV: Multiposition valve, IV: injection valve; MSP: multisyringe pump; HPLC: high performance liquid chromatograph. HC: holding coil. CC: communication channel. The inset illustrates two designs of stirred-flow chambers for μSPE.

recording of chromatogram peaks, and data processing using peak area measurements was performed automatically by a PC operated under the Chem Station Rev.10.01 software (Agilent).

Stirred-Flow Sorptive Microcolumns. Two stirred-flow sorptive microcolumn configurations, namely, the commercially available Mobicol cartridge (Mobicol M 1002, MoBiTec, Göttingen, Germany) placed in the horizontal position, and an in-house built cylindrical microcolumn in the upright position, were investigated as flow-through containers for handling of dispersed CNTs and CNFs.

The Mobicol column was furnished with luer-lock adaptors at both ends for press-fit connection to the flow network. The nominal column capacity was 1000 μL. A given amount of CNFs/CNTs was introduced (<30% column capacity) together with a small magnetic bar (5 mm long × 2 mm diameter), which were trapped inside by 10 μm-pore size polyethylene filters (MoBiTec) at both column ends.

The dedicated cylindrical chamber (1.0 cm height, 8 mm i.d.) designed to contain ≤80 mg CNTs/CNFs and a magnetic bar was constructed from polyetherimide (Ultem) to have a nominal capacity of ca. 500 μL. The bottom outlet of the chamber was equipped with a 10-μm polyethylene frit (MoBiTec) for efficient trapping of the nanoparticles while the solutions were allowed to flow freely. Metered volumes of sample and eluent were delivered sequentially on the top of the chamber for uptake of target species and retrieval of preconcentrated compounds, respectively. The CNT/CNF containers were placed on a magnetic stirrer actuated at 100 rpm during sample percolation. The sorptive preconcentration capacity of both stirred-flow reactor configurations for triazines in an automated mode was compared by resorting to 10 mg of CNFs.

Flow-Based Assembly. A multisyringe piston pump with programmable speed (MicroBu 4S, Crison Instruments, Alella, Barcelona, Spain) was used as a liquid driver for automation of solution handling and of stirred-flow sorptive microcolumn operations and for at-line coupling of CNT/CNF-based microextraction with LC. It was equipped with two high-precision bidirectional syringes (Hamilton, Switzerland), labeled as S1 and S2 in Figure 1, with a capacity of 5.0 mL each and connected in block to a 40 000-step motor. S1 and S2 contained both Milli-Q water and were used for fluid delivery and postcolumn remixing of the organic eluate with Milli-Q water prior to LC separation, respectively. A three-way solenoid valve (N-Research, Caldwell, NJ) was placed at the head of each syringe, enabling automatic connection with either the liquid reservoirs (OFF) or the flow network (ON). The multisyringe module was coupled to an eight-port multiposition valve (MPV, Valco Instruments, Houston, TX) operating as central processing unit. The MPV encompasses a central port and a communication channel (CC) that can be programmed to address each of the peripheral ports. A 45-position rack autosampler (Crison Instruments) was attached to MPV for automatic processing of up to 45 water samples and soil extracts. The external vial for eluate collection attached to port 3 in MPV is composed of a polypropylene syringe barrel of 2.5 mL capacity connected to the flow network

with a luer adapter for PTFE manifold tubing. The central port was connected via a 5.0 mL holding coil (HC, 1.5 mm i.d PTFE tubing) to S2 for fluidic handling. The MPV was connected via a 60 μL transfer line (0.8 mm i.d. PTFE) to a six-port rotary injection valve (IV, Valco) furnished in-loop with the stirred-flow CNT/CNF microcolumn.

Analytical Procedure. The automated analytical sequence of the SI-LC hyphenated setup for trace level assays of chlorotriazine herbicides in environmental waters and crude soil extracts involved in-line conditioning of the CNTs/CNFs, sample perfusion through the stirred-flow sorptive microcolumn, rinsing of carbon nanoparticles, sorbent drying, analyte elution, in-line modulation of eluate composition, and online injection of eluate into LC. The operational details of the overall procedure are described below.

Conditioning of CNTs/CNFs. Automatic sorbent conditioning was accomplished by consecutive delivery at 1.0 mL min^{-1} of 2.0 mL of methanol and 2.0 mL of Milli-Q water for water samples, or 4.0 mL of methanol and 4.0 mL of Milli-Q water for soil samples, followed by 2 mL of air so as to empty the sorptive microcolumn prior to sample loading.

Sample Loading. S2 was programmed to aspirate 100 μL of air (to prevent sample dispersion into the holding coil, HC) and 2.0 mL of water sample consecutively into HC followed by backward perfusion through the stirred-flow microcolumn at 1.0 mL min^{-1}. The loaded sample was pumped to the external collector and from there to waste. These operational steps were 5-fold repeated in order to handle a total sample volume of 10 mL. For soil extracts, a 1.0 mL of sample was perfused through the microcolumn followed by 1.0 mL of 5% (v/v) methanol at 1.0 mL min^{-1} to remove the coadsorbed matrix materials onto CNFs/CNTs. The injection valve was then switched to the inject position whereupon the external diaphragm pump (Laboport, 65 W, KNF lab, Freiburg, Germany) was activated for 10 min for drying of the CNTs/CNFs by compressed air.

Analyte Elution. Retrieval of preconcentrated triazine residues was effected with a metered volume of 470 μL of methanol at 0.5 mL min^{-1} followed by an air plug (100 μL) to prevent dispersion of the eluate at the carrier boundary through the container. In the course of the elution, the magnetic stirrer was turned off for efficient desorption of target species and minimization of losses of eluate within the CNT/CNF microcolumn. The overall eluate was collected in the external collector and thoroughly mixed by air bubbles for 30 s. Potential analyte carryover was overcome by thorough rinsing of the CNTs/CNFs with organic solvent in the conditioning step.

Automatic Analysis of Eluate. A metered air-segmented plug of eluate (170 μL) from the external collector was drawn into HC and then directed forward to merge with a Milli-Q water stream (provided by S1 at 1.0 mL min^{-1}) at a 1:1 ratio before transferring the entire composite plug into the LC loop. The LC injection valve was then activated and the LC gradient protocol (see above) initiated. The chromatographic separation was synchronized with the μSPE procedure, whereby a given water sample was analyzed while the ensuing one was being processed in the SI manifold. The automated operational sequence for a single sample lasted ca. 25 min for waters, thus matching the time frame of the chromatographic run and re-equilibration of the analytical column to the initial conditions, which amounted to 20−25 min alike. The μSPE column was filled with methanol overnight (or whenever not in use) for solvation and rejuvenation of sorptive CNTs/CNFs.

RESULTS AND DISCUSSION

Selection of Sorbent Nanomaterial and Eluent. Preliminary tests were undertaken to explore the analytical performance of distinct carbon nanoparticles, namely, MWCNTs, MWCNT-COOH, and CNFs as μSPE materials in a manual sorptive mode for the uptake of the overall chlorotriazines and metabolites thereof prior to LC assays. The packed cartridges contained 10 mg of carbon nanoparticles each and were furnished with glass wool and frit (10 μm) at both ends using a 1.0 mL polypropylene cartridge as a body column. These experiments were designed for the enrichment of 1.0 mL of triazine standard mixture containing 100 ng mL^{-1} each. Subsequently, the compounds retained on the cartridge were eluted with two batches of 500 μL each of organic solvent to ensure quantitative retrieval of analytes.

Experimental results revealed that regardless of compound polarity, early analyte breakthrough was detected in MWCNTs and MWCNT-COOH with losses ≤40% for the less hydrophobic dealkylated (DIA and DEA) metabolites. Conversely, absolute recoveries ≥95% (see Figure S1, Supporting Information) were obtained for the suite of investigated compounds whenever the same amount of CNFs was used instead. Compared with CNTs, CNFs feature larger chemically active surface area for sorption as a result of the cup-stacked configuration of the nanomaterial. Upon preliminary experiments concerning μSPE in combination to LC, leaking and accumulation of CNFs onto the LC precolumn was not at any instance detected in contrast to CNT-based extraction, because of significantly larger dimensions of the fibers.

Of the several eluents reported in the literature for elution of pesticides/herbicides from CNTs, including acetone, methanol, acetonitrile, ethyl acetate, dichloromethane, hexane, cyclohexane, and mixed solvents,[8,18,21,23−25] methanol and acetonitrile were compatible with the reversed-phase separation of triazines and afforded better absolute recoveries. However, two consecutive plug elutions were needed in the case of acetonitrile for quantitative retrieval of overall herbicides and band-broadening effects were detected for both dealkylated metabolites with injection volumes ≥240 μL in a 50% (v/v) eluate/water medium. Under the same experimental conditions, quantitative elution of overall triazine residues was proven feasible using a single MeOH plug, with RSDs < 4% against 5−8% in acetonitrile (see Figure S1), without detecting band-broadening of peaks up to an injection volume of 340 μL in a 50% eluate/water medium. In the coupling of CNF-based sample pretreatment with LC in a fully automated mode, the larger the eluate volume injected the better are the enrichment factors. Pure methanol was selected as eluent for the remainder of the work accordingly.

Configuration of the Flow-through CNF-Based Sorptive Column. Carbon nanoparticles are characterized by a marked tendency to aggregation, which negatively affects the sorptive uptake of target species whenever integrated in flow systems as permanent packed columns. This is a result of the decrease in surface area and undue flow resistance within the flow network in the sample loading and elution stages as described earlier in the literature.[1,9,10] To overcome this drawback while avoiding nanomaterial functionalization, coating or decoration using inert nanoparticles, two new column configurations for expedient handling of CNFs, as detailed in Experimental Section, have been herein explored. CNF entanglement and bundling were minimized by resorting to μSPE with mechanically dispersed sorbent in the course of sample loading. Scanning electron

micrographs revealed no appreciable mechanical splitting of CNFs under the experimental conditions detailed above.

In the horizontally placed Mobicol microcolumn, the mixing pattern of CNFs in the suspending media lacked reproducibility because of the irregular shape and narrow ends of the reactor. Furthermore, this arrangement was proven unable to endure the build-up of backpressure in sample percolation, and leaking was frequently observed.

The absolute recoveries of the stirred-flow cylindrical microcolumn in upright configuration ranged between 96 to 100% for the suite of chlorotriazines and metabolites thereof against 80−89% in the Mobicol column. The superior performance of the dedicated microcolumn for flow-through sorptive preconcentration is a consequence of the better hydrodynamics of this configuration for efficient dispersion of CNFs within the flowing solutions.

Effect of the Amount of CNFs on Triazine Retention and Elution Efficiencies. The reliability in handling varied amounts of CNFs in the dedicated microcolumn configuration was investigated in terms of uptake and retrieval of chlorotriazines and of automated coupling to LC. Notwithstanding the fact that increasing masses of CNFs up to 80 mg were proven not to cause

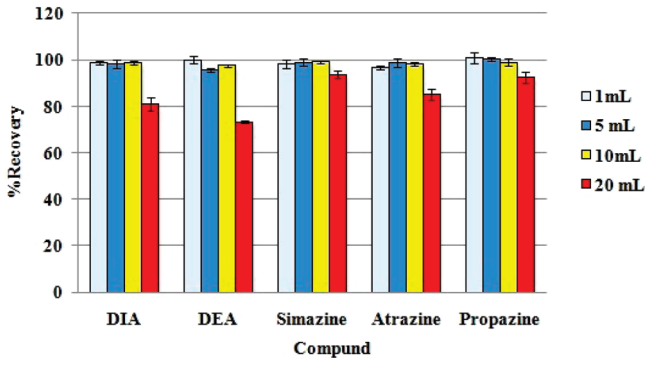

Figure 2. Investigation of the influence of the sample volume on analyte recovery in the stirred-flow CNF microcolumn arrangement for the preconcentration of a given amount of chlorotriazines (100 ng each) using 1, 5, 10, and 20 mL of 100, 20, 10, and 5 ng mL^{-1}, respectively. Analytes were eluted by 470 μL of methanol at 0.5 mL min^{-1}. Error bars are given as one standard deviation (n = 3).

an increase of pressure drop in the devised flow-through configuration and afford absolute recoveries close to 100% (namely, 98−104%), the eluent volumes needed to be increased from 470 up to 4000 μL (see Figure S2, Supporting Information). In fact, when carbon sorbents are used in SPE procedures, poor recoveries for organic compounds have been described in the literature because of difficulties in recovering trapped species.[26] This accrues with triazine residues because of strong $\pi-\pi$ stacking interactions between the electron-rich rings of CNFs and the electron-deficient aromatic heterocyclic rings of triazines. Large eluent volumes of pure solvents are ill-suited for direct injection into reversed-phase LC because of acute band-broadening effects for most polar triazines and column flooding as well. Online coupling of the CNT/CNF-packed column to LC is thus cumbersome, as column-switching and heart-cut elution protocols are deemed inappropriate for ensuring sufficient enrichment factors. This accounts for the fact that the majority of flow-through CNT-packed columns or impregnated membranes connected to column separation or detection systems[1,10,12,13] involved functionalized materials to retain target species by coulomb forces in lieu of noncovalent hydrophobic interactions. Hence, elution was therein facilitated by pH change or ionic-strength gradient. To alleviate this shortcoming, we herein propose a versatile approach for automated manipulation of variable volumes of eluent as per assay needs, capitalizing on at-line coupling of the CNF-based preconcentration setup to LC (using an external container attached to the MPV, see Figure 1), followed by in-line dilution of a 170 μL eluate plug at a 1:1 MeOH/H_2O ratio. The requirements for LC separation with efficient band focusing of DIA and DEA are thus met.

In our particular application, the conjunction of 10 mg of CNFs and 470 μL of MeOH was proven most suitable for processing residues of triazines at submicrogram per liter levels in environmental waters and crude soil extracts as well. Elution was undertaken under quiescent (nonstirring) conditions to prevent dispersion of analyte-bearing CNFs in the column bulk medium, which otherwise would give rise to undue broadening of the elution profiles and the requirement of increasing eluent volumes for quantitative retrieval of analytes.

Effect of Sample Loading Flow Rate and Sample Volume on Triazine Enrichment. The sample volume and perfusion

Table 1. Analytical Performance of the SI-CNF-LC Method for Determination of Chlorotriazines and Metabolites Thereof Using Different Sample Volumes

sample volume	compound	regression equation	determination coefficient (r^2)	linear range (ng mL^{-1})	enrichment factor	absolute recovery (%)	RSD (%)	LOD[c] (ng mL^{-1})	LOQ[d] (ng mL^{-1})
10 mL[a]	DIA	$y = 11.6x + 0.5$	0.9998	0.1−10	20.4	96	0.5	0.02	0.08
	DEA	$y = 25.5x - 0.3$	0.9996	0.1−10	20.6	97	1.2	0.004	0.01
	simazine	$y = 23.4x - 1.0$	0.9985	0.1−10	21.1	99	1.7	0.01	0.04
	atrazine	$y = 25.5x - 0.9$	0.9990	0.1−10	21.1	99	1.4	0.02	0.05
	propazine	$y = 16.5x - 0.9$	0.9985	0.1−10	20.0	94	1.8	0.03	0.09
1 mL[b]	DIA	$y = 0.04x - 0.02$	0.9976	10−100	2.1	98	0.5	0.53	1.78
	DEA	$y = 0.08x + 0.03$	0.9959	10−100	2.1	99	0.6	0.30	0.99
	simazine	$y = 0.08x + 0.10$	0.9988	10−100	2.2	102	0.6	0.20	0.67
	atrazine	$y = 0.08x + 0.12$	0.9968	10−100	2.1	98	0.8	0.17	0.58
	propazine	$y = 0.05x + 0.18$	0.9932	10−100	2.0	96	0.6	0.21	0.70

[a] Intended for water assays using external calibration. Calibration standards: 0.1, 0.5, 1.0, 2.0, 5.0, and 10 ng mL^{-1}. [b] Intended for cleanup of soil extracts using linuron as internal standard. Calibration standards: 5.0, 10, 20, 50, and 100 ng mL^{-1}. [c] LOD was calculated as S/N = 3 on the basis of the analytical signals at the 0.5 and 5.0 ng mL^{-1} levels for sample volumes of 10 and 1 mL, respectively. [d] LOQ was calculated as S/N = 10 on the basis of the analytical signals at the 0.5 and 5.0 ng mL^{-1} levels for sample volumes of 10 and 1 mL, respectively.

flow-rate through the stirred-flow μSPE column are regarded as relevant physical variables for concentration and screening/determination of triazine herbicides at environmentally relevant levels in a timely manner.

Investigation of analyte breakthrough for 10 mg of CNFs was performed in a dynamic mode by loading a given amount of target chlorotriazines (100 ng each) in increasing volumes of mixed aqueous standards from 1 to 20 mL (see Figure 2). No pre-elution effect was observed for any of the target compounds up to 10 mL. Loss of the more polar metabolites DIA and DEA by $\geq$20% was however encountered for a sample percolation volume of 20 mL. A sample volume of 10 mL was thus selected for the remainder of the work and real sample assays.

Because of strong affinity of CNFs to the triazine rings, fast sorption is expected. The uptake of 10 mL of mixed standard at the 10 ng mL^{-1} level was investigated over the range 1.0−3.0 mL min^{-1}. The absolute recoveries of the overall analytes were close to 100% at 1.0 mL min^{-1}. Increasing to 1.5 and 2.0 mL min^{-1} led to 10% decrease in recoveries for DIA, DEA, and simazine and 5% for atrazine and propazine when compared to 1.0 mL min^{-1}. Recoveries of the less hydrophobic dealkylated metabolites, namely, DIA and DEA, decreased down to 80% when increasing the sample loading flow rate to 3.0 mL min^{-1}. It has been previously reported that reversed-phase copolymeric materials with hydrophobic/hydrophilic balance showcase superior retention efficiency for the more polar triazine residues.[27] At the expense of the concentration efficiency, the sample loading flow rate was affixed to 1.0 mL min^{-1} for method validation and sample assays aimed at ensuring maximal enrichment factors and absolute recoveries, yet sample loading at 2.0 mL min^{-1} might be still applied for fast screening of chlorotriazines in environmental samples.

Method Validation and Application to Real Environmental Samples. Under the selected physical and chemical variables detailed in the foregoing sections, the analytical performance of the hyphenated SI-CNF-LC method was investigated in terms of dynamic linear ranges, enrichment factors, absolute recoveries, repeatability, and sensitivity for further assays of surface and ground waters and soil extracts for determination of parent triazines and metabolites at environmentally relevant concentrations. The figures of merit are summarized in Table 1.

Quantification of target herbicides was undertaken by exploiting six-level calibration plots with correlation coefficients >0.9966. Concentration ranges spanned over 2 orders of magnitude, namely, 0.1−10 or 10−100 ng mL^{-1} as per the standard volume percolated, that is, 10 or 1 mL, respectively. Internal standardization using linuron at the 100 ng g^{-1} level and detection at 220 nm was utilized in soil assays.

Absolute recovery percentages of the target chlorinated residues were calculated as the ratio between peak areas in the SI-CNF-LC method and those obtained from direct chromatographic injection of 340 μL containing an equivalent mass of standard in a 1:1 MeOH:H_2O medium. Absolute recoveries of triazine herbicides in the stirred-flow column configuration with dispersed CNFs were almost 100% regardless of the slight differences in lipophilicity among parent and dealkylated triazines.

Method repeatability was expressed as the precision (RSD) obtained from eight consecutive assays of a 10 mL mixed standard solution at the 2 ng mL^{-1} level (or 1.0 mL at the 20 ng mL^{-1} level) using a single CNF column. Relative standard

Table 2. Concentrations and Relative Recoveries of Target Chlorotriazines and Metabolites in Untreated Environmental Waters[a]

	spiked groundwater						spiked creek water						spiked tap water					
	spike level, (ng mL^{-1})						spike level, (ng mL^{-1})						spike level, (ng mL^{-1})					
	0.5		2.0				0.5		2.0				0.5		2.0			
compound	found	%rec	found	%rec	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	found	%rec	found	%rec	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	found	%rec	found	%rec	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
DIA	0.50 ± 0.02	100.0	1.92 ± 0.02	96.0	0.03	0.09	0.48 ± 0.01	96.0	1.97 ± 0.01	98.5	0.03	0.10	0.50 ± 0.01	100.0	1.95 ± 0.03	97.5	0.02	0.07
DEA	0.48 ± 0.02	96.0	2.03 ± 0.04	101.5	0.03	0.09	0.49 ± 0.02	98.0	1.99 ± 0.02	99.5	0.02	0.07	0.48 ± 0.01	96.0	1.98 ± 0.02	99.0	0.02	0.06
simazine	0.49 ± 0.01	98.0	2.01 ± 0.02	100.5	0.02	0.07	0.50 ± 0.01	100.0	1.99 ± 0.02	99.5	0.03	0.08	0.51 ± 0.02	102.0	1.99 ± 0.02	99.5	0.02	0.06
atrazine	0.51 ± 0.02	102.0	2.07 ± 0.04	103.5	0.02	0.07	0.51 ± 0.02	102.0	1.98 + 0.02	99.0	0.02	0.07	0.49 ± 0.02	98.0	1.99 ± 0.04	99.5	0.02	0.07
propazine	0.47 ± 0.01	94.0	1.97 ± 0.02	98.5	0.03	0.10	0.49 + 0.01	98.0	1.99 ± 0.01	99.5	0.03	0.09	0.50 ± 0.02	100.0	2.02 ± 0.04	101.0	0.02	0.08

[a] Results are given as the average of three replicate assays ± SD, rec: mean recovery

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Table 3. Concentrations and Relative Recoveries of Target Chlorotriazines and Metabolites in Crude Soil Extracts[a]

	soil #1							soil #2						
	spike level, (ng g^{-1})							spike level, (ng g^{-1})						
		20		50					20		50			
compound	0	found	%rec	found	%rec	LOD (ng g^{-1})	LOQ (ng g^{-1})	0	found	%rec	found	%rec	LOD (ng g^{-1})	LOQ (ng g^{-1})
DIA	nd	17 ± 2	85.0	44 ± 3	88.0	4.0	13.4	nd	17 ± 1	85.0	43 ± 5	86.0	4.3	14.4
DEA	nd	17 ± 3	85.0	47 ± 2	94.0	4.1	13.8	nd	16.7 ± 0.5	83.5	44 ± 3	88.0	4.0	13.4
simazine	8 ± 2	27.3 ± 0.3	97.5	59 ± 5	101.7	1.6	5.4	nd	18.4 ± 0.6	92.0	49 ± 3	98.0	1.8	5.9
atrazine	nd	19.8 ± 0.8	99.0	49 ± 4	98.0	1.4	4.5	nd	20.9 ± 0.4	104.5	48 ± 3	96.0	1.3	4.3
propazine	nd	21 ± 1	105.0	48.2 ± 0.9	96.4	2.7	9.0	nd	17 ± 2	85.0	48 ± 2	96.0	2.6	8.6

[a] Results are given as the average of three replicate assays ± SD, nd: not detected. rec: mean recovery.

deviations ranging from 0.5 to 1.8% regardless of sample volume were significantly better than those recently reported for batchwise activated carbon or MWCNT-based sorptive microextraction of triazines in waters with RSDs > 5%.[21,23,28]

Detection (LODs) and quantification (LOQs) limits were calculated on the basis of the signal-to-noise ratio (S/N) criteria of 3 and 10, respectively,[29] for analysis of 10 mL spiked Milli-Q water at the 0.5 ng mL^{-1} level for water or 1 mL standard at the 20 ng mL^{-1} level for soil analysis (see Table 1). The background noise was estimated as the peak-to-peak baseline signal close to the analyte peak. To evaluate the dependence of noise upon matrix complexity, LODs and LOQs were calculated for individual water and soil samples assayed (see Tables 2 and 3). LODs and LOQs for the various environmental waters ranged from 0.02 to 0.03 and 0.06 to 0.10 ng mL^{-1}, respectively (see Tables 2 and 3). It should be borne in mind that maximum allowed concentrations (MAC) of atrazine and simazine in surface waters are set to 2.0 and 4.0 ng mL^{-1}, respectively, by EU Water Framework Directive 2008/105/EC[16] and <0.1 ng mL^{-1} in tap waters by Directive 98/83/EC[30] including overall parent compounds and metabolites. This in turn demonstrates that the hyphenated SI-CNT-LC method fully meets the requirements specified in current legislation for determination of triazines in water bodies using merely a 10 mL sample as opposed to >100 mL in batchwise methods involving carbon nanomaterials.[8,21,23,25]

To explore the applicability and reliability of the proposed hyphenated method for preconcentration of chlorotriazines with concomitant sample cleanup in environmental assays, samples of variable matrix complexity, namely, environmental waters and soil extracts, were processed with minimum prior sample treatment. Because of the lack of certified reference materials containing the target triazines (concentrations of atrazine and simazine in CRM 606 are not certified any longer), environmental waters and agriculture soils were doped with the overall analytes at the 0.5 and 2.0 ng mL^{-1} levels for water (≤MAC for atrazine and simazine in Directive 2008/105/EC) and 20 and 50 ng mL^{-1} for soils, and analyzed in triplicate for assessment of method trueness. Relative recoveries in the automatic SI assembly for the suite of analyzed herbicides in untreated environmental waters and crude soil samples ranged from 94 to 103.5% and 83.5 to 105.0%, respectively. Superior recoveries were herein encountered as compared to earlier batchwise CNT-SPE procedures for determination of chlorotriazines in surface waters with recovery values of several target analytes within the range of 80−85%.[18,25,31] The μSPE column is proven to be reused for more than 3 days with negligible drop in retention efficiency when handling 120 mL water samples per day provided that the CNFs are solvated overnight or whenever not in use with pure MeOH.

Recoveries of the most polar dealkylated metabolites in soil extracts and assay precision were slightly deteriorated because of partial stripping of analytes when rinsing the sorptive nanoparticles with a minute volume of 5% MeOH to remove concomitantly sorbed matrix ingredients. Peak overlap with interfering species was also in some instances observed. However, improved recoveries were still afforded with respect to a recent work on the use of MWCNTs for batchwise soil assays with recoveries of 72−89% for atrazine, DIA, and DEA.[23] Chromatograms in soil assays as obtained from the at-line hyphenated SI-CNF-LC assembly are given in Figure S3, Supporting Information.

Earlier researchers exploiting packed MWCNTs for batchwise preconcentration and cleanup of pesticides and herbicides in soils[23,24] recommended single use of SPE cartridges because of strong sorption properties toward concomitant soil components. A major asset of the proposed assembly is the potential reuse of the dispersed CNFs without loss of retention efficiency for a given number of assays (as per soil matrix complexity) provided that the sorptive nanoparticles are regenerated with 4.0 mL of MeOH between runs.

CONCLUSION

The proof-of-concept of a dedicated stirred-flow microcolumn configuration for handling of dispersed carbon nanomaterials as sorptive surfaces in automatic flow-mode with minimum nanoparticle agglomeration and negligible pressure drop has been demonstrated for μSPE and cleanup of triazine herbicides of differing polarity in both environmental waters and soil extracts. To the best of our knowledge, this is the first report wherein carbon nanoparticles have been integrated in automated flow-systems for soil assays.

The beauty of the devised assembly is that nanoparticles are to be used as-purchased with no need for cumbersome immobilization procedures to afford composite core−shell nanoparticles or decorated membranes. Not the least, multistage μSPE procedures including sorbent regeneration have been fully automated by resorting to programmable flow inherent to sequential injection analysis. The versatile at-line interface to LC fosters

independent optimization of the CNF-based sample processing regardless of the eluent volume for quantitative stripping of preconcentrated species. The hyphenated analyzer provides sufficient sensitivity and reliability for determination of triazine herbicides at concentration levels below those specified by current legislations for human water consumption and surface waters.

This work results in significant progress toward the development of miniaturized and simplified sample handling methodologies with green chemical credentials because of the use of minute amounts of nanomaterials and organic solvents contrarily to manual SPE cartridges. Research work in our lab is underway to expanding the proposed flow-analyzer for simple handling of other nanoparticles or composites within μSPE procedures for assays of priority pollutants in environmental matrixes and for bioassays of endogenous organic species and xenobiotics as well.

■ ASSOCIATED CONTENT

Supporting Information. Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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CHAPTER 9

On-line coupling of bead injection-lab-on-valve analysis to gas chromatography (BI-LOV-GC): Application to the determination of trace levels of polychlorinated biphenyls(PCBs) in solid waste leachate samples

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Online Coupling of Bead Injection Lab-On-Valve Analysis to Gas Chromatography: Application to the Determination of Trace Levels of Polychlorinated Biphenyls in Solid Waste Leachates

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Online sorptive preconcentration exploiting renewable solid surfaces, so-called bead injection (BI), in the miniaturized lab-on-valve (LOV) platform is for the first time hyphenated to gas chromatography (GC) for automated determination of trace level concentrations of organic environmental pollutants. Microfluidic handling of solutions and suspensions in LOV is accomplished by programmable flow with a multisyringe flow injection (MSFI) setup. The method involves the incorporation of minute amounts (3 mg) of reversed-phase copolymeric beads with hydroxylated surface (Bond Elut Plexa) into the channels of a poly(ether imide) LOV microconduit, thus serving as a transient microcolumn packed reactor for preconcentration of organic species. The analyte-loaded beads are afterward eluted with 80 μL of ethyl acetate into a rotary injection valve and subsequently introduced via an air stream into the programmable-temperature vaporizer (PTV) injector of the GC. The used beads are then backflushed and delivered to waste. The GC separation and determination is synchronized with the preconcentration steps of the ensuing sample. The potentials of the devised BI-LOV–GC assembly with electron capture detector for downscaling and automation of sample processing were demonstrated in the determination of polychlorinated biphenyls in raw landfill leachates and a leachate containing the Aroclor 1260 congener mixture. By sampling 12 mL of leachates to which 50 vol % methanol was added to minimize sorption onto the components of the flow network, the automated analytical method features relative recovery percentages >81%, limits of quantification within the range of 0.5–6.1 ng L^{-1}, relative standard deviations better than 9% at the 50 ng L^{-1} level, and 25-fold decrease in cost of solid-phase extraction (SPE) consumables as compared with online robotic systems or dedicated setups.

Solid-phase extraction (SPE) has become the preferred routine sample preparation technique for the determination of organic pollutants[1] because of the advantageous features over liquid–liquid extraction and improved capacity and robustness as compared to liquid-phase or solid-phase microextraction counterparts. Yet, sample preparation accounts for over 60–80% of the total analysis time and normally is the main contributor to analytical uncertainty.[2–4] Thus, automation of sample preparation is of great value in order to maximize throughput and minimize costs, time, and analyst risks due to chemicals exposure. In this context, online coupling of SPE to chromatography represents the automation milestone in water analysis of organic pollutants, as the overall analytical protocol can be fully mechanized. Although the online SPE–liquid chromatography (LC) coupling is currently well established, SPE–gas chromatography (GC) hyphenation via mechanized flow-based approaches is far less common.[2] Actually, as reviewed by Hyötyläinen and Riekkola,[3,4] most applications date back to the 1990s, and research on this topic in the last years is quite scarce. This observation can be attributed to the fact that SPE columns for water analysis should be gently dried prior to elution and the final eluate volume needs to be reduced prior to introduction into the GC capillary column, most often by an on-column or programmable-temperature vaporizer (PTV) injector, to avoid column overload.[2–5] As a result, the SPE–GC coupling is technically more complex and difficult to optimize. Online analyzers, so-called Prospekt-2 (Spark Holland, The Netherlands), for automated SPE are currently available,[6–9] yet this robotic system requires the modification of the PTV injector, whereby the GC can merely be used for analysis in

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combination with the robotic SPE analyzer. Prospekt-2 setups allow the renewal of the sorbent cartridge in each analytical cycle. Most researchers have, however, decided to regenerate the sorbent material and reutilize it in several assays as a consequence of the elevated costs of these dedicated cartridges.[6−8]

Indeed, the Achilles's heel of online SPE−GC analyzers described in the literature up to date is the usage of a permanent sorbent column attached to the flow setup, which deteriorates the ruggedness of the system and calls for extensive operational maintenance. In fact, it has been reported that the increasing of back-pressure in real-life sample analyses caused by the progressive tighter packing of the sorbent beads when reusing the material for a given number of assays hinders the effective drying of the sorbent after sample loading.[6] Further, the reuse of columns might result in deterioration of peak shapes of target analytes in GC,[6] most likely due to the concomitant adsorption of matrix ingredients. Hence, online SPE−GC analyzers have been merely utilized for automated determination of trace level of organic pollutants in simple matrixes, e.g., surface waters.[6−8] Analyte carryover effects over 5−15% have been also observed in copolymeric phases between sample runs, hereby tedious cleansing procedures should be applied.[6]

The difficulties associated with flow resistance can be alleviated to a certain extent via various approaches, including the performance of the elution sequence in the backflush mode.[10] Yet, the malfunctions of the sorbent surfaces themselves are not addressed by these means. Therefore, a superb alternative for eliminating any problems associated with the changes of the surface properties of the sorbent materials and/or the creation of flow impedance in a column reactor is to employ a surface renewal scheme, that is, the so-called bead injection (BI) procedure.[11,12] Here, the contents of the packed column are automatically renewed for each analytical run. Such a scheme is readily downscalable in the lab-on-valve (LOV) platform,[13−18] which has opened up a host of prospects within the environmental analytical field.

The microconduit LOV unit is a single monolithic structure mounted atop of a multiposition valve of a sequential injection network. The LOV approach should be viewed as a judicious advance toward the automation of microfluidic handling of samples and sorbent materials within integrated microbore units.[16] In short, packed column reactors for microscale SPE[14,15,18,19] are in situ generated by aspirating beads with particular surface characteristics and particle sizes, advantage being taken of the fact that the sorbent can be manipulated exactly as when handling liquids. The solid entities can even be automatically transported between different column positions within the LOV, their retention within the columns being facilitated by fitting the column positions with appropriate stoppers or frits, which will keep hold of the beads, yet allow solutions to flow freely. Following sample loading and cleanup protocols, appropriate eluents can be aspirated, and the eluate propelled to the detection device, as sandwiched by air or immiscible liquid segments in order to preserve its integrity.[17] The microfabricated channel system is amenable to incorporate detection facilities, that is, optical devices (namely, diode-array spectrophotometers, charged-coupled devices (CCDs), laser-induced spectrofluorimeters or luminometers)[15,16,20] or electrochemical detectors,[21,22] but also to admit conventional-sized peripheral devices. Hence, LOV might be hyphenated to a plethora of modern detection techniques/analytical instruments, such as electrothermal atomic absorption spectrometry,[19,23−25] cold-vapor atomic absorption spectrometry,[26] electrospray ionization mass spectrometry,[27,28] atomic fluorescence spectrometry,[29−31] inductively coupled plasma mass spectrometry,[32] and most importantly for determination of organic contaminants, to separation systems, such as capillary electrophoresis[33−35] or high-performance liquid chromatography.[36] However, the coupling of LOV with GC has not been to the best of our knowledge yet reported.

This work aims at appraising the analytical performance of BI-LOV as a front end to large volume injection GC exploiting multisyringe flow pumping for execution as demanded in environmental assays of appropriate sample pretreatment, such as matrix isolation and analyte preconcentration prior to introducing the analytes optimally into the GC apparatus for separation. The considered case study herein has been the determination of polychlorinated biphenyls (PCBs) in complex environmental matrixes, that is, solid waste leachates. Landfill leachate is a major source of water and soil pollution with PCBs if not properly monitored.[37,38] Moreover, no method has been published to date dealing with online SPE−GC determination of PCBs in solid waste landfill leachates or environmental waters.

MATERIALS AND METHODS

Chemicals and Stock Solutions. A mix standard containing the PCB-28, PCB-52, PCB-101, PCB-118, PCB-153, PCB-138, and PCB-180 congeners at a concentration level of 10 mg L^{-1} each in isooctane was obtained from Absolute Standards (Hamdem, CT). A solution of 100 mg L^{-1} of PCB-166, employed as internal standard (IS), in isooctane was also from Absolute Standards. A standard solution of 1000 mg L^{-1} of Aroclor 1260 in isooctane was purchased from Supelco (Bellefone, PA). Stock standards were diluted to the desired concentration in acetone for sample spiking or in isooctane or ethyl acetate for direct injection into GC.

Reversed-phase bead material explored for BI-SPE analysis involved copolymeric Oasis HLB (30 μm, Waters, Mildford, MA), copolymeric Lichrolut EN (40−120 μm, Merck, Darmstadt, Germany), copolymeric Bond Elut Plexa (40−55 μm, Varian, Palo Alto, CA), and spherical octadecyl chemically modified silica Upti-

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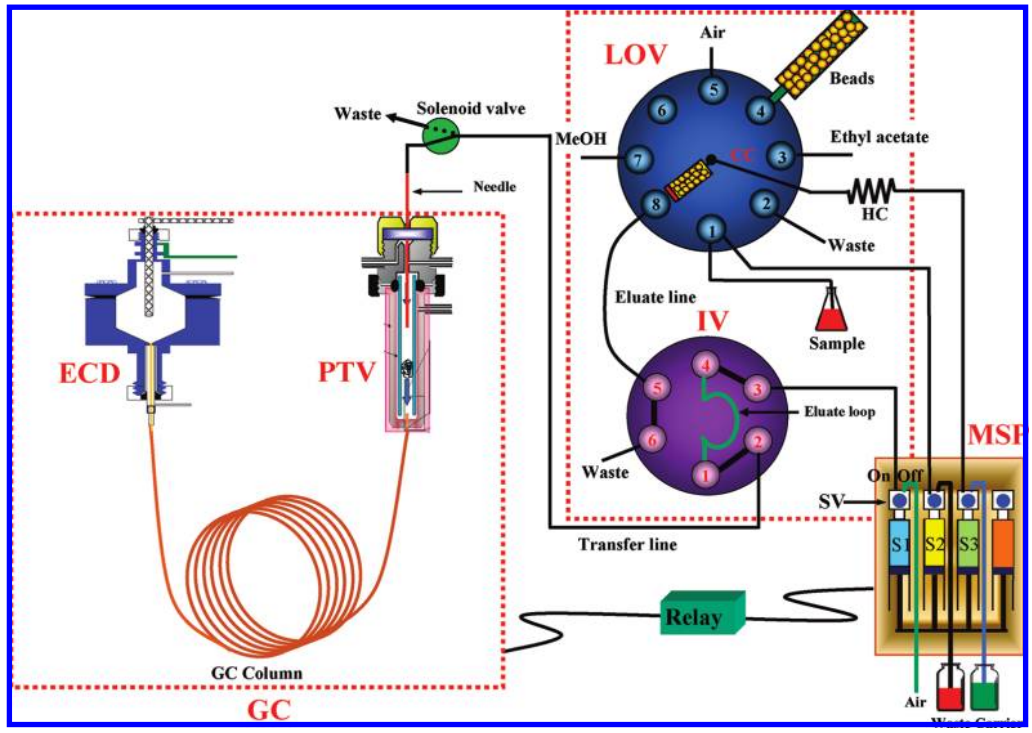

Figure 1. Schematic illustration of the multisyringe flow-based bead injection lab-on-valve setup hyphenated to large volume injection gas chromatography for preconcentration and determination of trace level concentrations of PCBs in solid waste leachates: LOV, lab-on-valve; IV, injection valve; MSP, multisyringe piston pump; HC, holding coil; PTV, programmed temperature vaporizer; SV, solenoid valve; GC, gas chromatograph; ECD, electron capture detector.

Clean C18 (50 μm, Interchim, Montluçon, France). Suspensions of 1:5 (w/v) of the reversed-phase beads were prepared in pure methanol.

Ultrapure water (18.2 MΩ cm) was obtained from a Milli-Q water generator (Millipore, Billerica, MA). Methanol for GC residue analysis (99.9%), acetone (99.8%), isooctane for GC residue analysis (99.9%), ethyl acetate (99.5%), and disodium ethylenediaminetetraacetic acid (EDTA) were all purchased from Scharlau (Barcelona, Spain). Toluene (99.5%) was supplied by Panreac (Barcelona, Spain).

Samples. Solid waste landfill leachate samples were collected in July 2008 in glass bottles and stored refrigerated. Two samples (nos. 1 and 3) were collected on different days from an urban waste landfill located in Santa Margalida (Mallorca, Illes Balears, Spain). Sample no. 2 was collected from a landfill of bulky and electronic equipment in Manacor (Mallorca). Before analysis, samples were filtered through a 0.45 μm cellulose filter and diluted to 50% (v/v) with methanol as an organic modifier to prevent adsorption of PCBs on the PTFE tubing of the flow manifold and glassware as well; otherwise PCBs could not be recovered quantitatively, and cross-contamination might occur. Sample no. 2 was also modified with 0.1% (w/v) disodium EDTA and 0.75% (v/v) concentrated ammonia to prevent precipitation of metal species.

Multisyringe Flow Injection Lab-on-Valve System. A multisyringe piston pump (MSP) with programmable speed (MicroBU 2030, Crison, Alella, Spain) equipped with three high-precision bidirectional syringes (S1, S2, and S3) (Hamilton, Switzerland) connected in block to a single stepper motor, was utilized as a multiple fluid driver. S1 and S3 with a capacity of 10.0 and 5.0 mL, respectively, contained air and the carrier solution (50% (v/v) methanol/water), respectively. S2 with a capacity of 5.0 mL served for rinsing the sampling line between consecutive samples or standards. A three-way solenoid valve (SV) (N-Research, Caldwell, NJ) was mounted atop of each syringe, enabling the communication with the liquid reservoirs or atmosphere in the OFF position, or with the flow manifold whenever activated to ON. The flow network was built from PTFE tubing of 0.8 mm i.d., excepting the 285 cm long holding coil (HC), which was made from 1.5 mm i.d. PTFE tubing. The multisyringe flow injection bead injection lab-on-valve (MSFI-BI-LOV) assembly hyphenated to PTV-GC is schematically illustrated in Figure 1.

The dedicated LOV microconduit (Sciware, Palma de Mallorca, Spain), fabricated from poly(ether imide) (Ultem) for chemical resistance to a wide range of organic solvents and encompassing eight integrated microchannels (1.2 mm i.d./14.0 mm length, excepting the bead reactor channel made of 1.7 mm i.d.), was mounted atop of an eight-port multiposition selection valve (Valco Instruments, Houston, TX). The central port of the integrated LOV sample processing unit, connected to S3, is made to address the

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peripheral ports of the unit (1−8), for sequential aspiration of the various constituents for the BI-based SPE process, via the central communication channel (CC) in the selection valve. One of the LOV channels (port 8) served as a microcolumn position for the renewable beads. To contain the sorbent within the cavity of the LOV microbore module and prevent them from escaping, the outlet of the column was furnished with a polyethylene frit of 10 μm (Mo Bi Tec, Göttingen, Germany). The suspension of reversed-phase beads was contained in a 1.0 mL plastic syringe, which was mounted vertically on port 4 of the integrated microsystem. The eluent and cleansing solvent reservoirs were attached to peripheral ports 3 and 7, respectively, whereas ports 2 and 5 were employed for sorbent disposal after each analytical assay and air aspiration for liquid segmentation, respectively. The specially designed dual channel (port 1) was utilized for sample introduction into the flow system, the outgoing channel being connected to S2, thereby permitting a thorough rinsing of the sampling tubing between samples to prevent cross-contamination effects. For quantitative injection of a metered eluate zone, a rotary injection valve (IV, Valco) furnished with a 150 μL eluate loop was connected at the outlet of the BI-SPE column. The LOV unit and the injection valve were connected via a 100 μL PTFE tubing. The 40 cm long transfer line to GC ends with a stainless steel capillary tubing of 6 cm × 127 μm i.d./794 μm o.d. (Supelco, Bellefonte, PA) permanently mounted in the PTV injector. A discrete solenoid valve was implemented within the transfer line for ease of rinsing of the transfer line between assays.

The operational procedures for the multisyringe flow-based LOV analyzer were fully computer-controlled by the software package AutoAnalysis 5.0 (Sciware) based on dynamic link libraries (DLLs). In our particular configuration, the principal protocol of the software was loaded with custom-built DLLs designed for the automatic control of the multisyringe pump and selection and injection valves. The multisyringe module is furnished with four digital outputs, each capable of providing 12 V/0.5 A. One of the digital outputs is connected to a relay, which is utilized for activation of GC via the Autoanalysis 5.0 software following injection of eluate into the PTV interface.

Gas Chromatography. Separation and detection of PCBs was performed with a CP-3800 gas chromatograph equipped with full electronic flow control (Varian, Walnut Creek, CA). The GC is composed of a 1079 PTV injector operating under solvent vent mode, a CP-Sil 8 capillary column (30 m × 0.25 mm × 0.25 μm, 95% methyl/5% phenyl polysiloxane, Varian), and an electron capture detector (ECD). Large volume injection was carried out into a glass wool packed gooseneck-type liner (54 mm × 3.4 mm i.d. × 5 mm o.d.) using the following temperature program: The temperature was initially set to 70 °C (held for 1.5 min), then increased to 280 at 150 °C min^{-1} (held for 10 min), and finally returned to initial temperature. The split valve was kept open for 1.5 min with a split ratio of 60 (purge flow of 78 mL min^{-1}) for solvent elimination, whereupon it remained closed for 3.0 min to proceed with the transfer of analytes into the GC capillary column. The column temperature program involved a first step of 60 °C (held for 5 min), increased to 170 °C (held for 10 min) at 30 °C min^{-1}, and finally raised to 250 °C (held for 5 min) at 3 °C min^{-1}. He (99.999%) was employed as carrier gas. Just before transfer of the liquid eluate into the PTV injector, the He pressure was automatically reduced to 1.0 psi (6.9 kPa) using the pressure pulse option of the GC instrument, in order to ensure the quantitative and reproducible introduction of the eluate into the PTV injector via the multisyringe flow setup. Subsequently, the inlet pressure was automatically increased to ensure a constant flow of 1.3 mL min^{-1}. The ECD was kept at 300 °C with a makeup of 29 mL min^{-1} of N_2 (99.999%). Setting and control of GC parameters, data acquisition, and processing were conducted using the Galaxie software package (Varian). Both Autoanalysis 5.0 and Galaxie softwares were installed in the same PC, whereby the flow setup and GC could be controlled simultaneously by a single PC.

Analytical Procedure for Automated BI-LOV Preconcentration and Determination of PCBs. The operational details of the BI-LOV−GC method based on flow programming for the determination of trace level concentrations of PCB congeners using reversed-phase copolymeric beads (Bond Elut Plexa) as sorptive media are detailed in the Supporting Information (Table SI-1). A complete measuring cycle runs through a given number of steps, namely, bead packing and conditioning, sample loading, drying of sorbent by an air purge, analyte elution, transfer of eluate into the PTV injector, and finally bead disposal and system conditioning.

The basic features of the analytical procedure can be summarized as follows:

Step 1: Bead Packing into LOV and Conditioning. S3 is set to aspirate consecutively 300 μL of air, 300 μL of methanol, and finally 300 μL of ethyl acetate into HC. A metered portion of the copolymeric bead suspension is next aspirated slowly (viz., 0.5 mL min^{-1}) into HC. The central port is then connected to port 8, and S3 is programmed to propel the sequentially aspirated plugs into the microcolumn channel. During this step, the beads are transferred to the column and moistened consecutively with ethyl acetate, methanol, and carrier (methanol/water (1:1)) for sorbent preconditioning.

Step 2: Sample Loading and Bead Drying. To prevent dispersion of the sample segment into the carrier solution, the LOV central port is directed to aspirate 500 μL of air from port 5 followed by aspiration of 4.0 mL of sample through port 1, which volume is stored into HC, prior to be being pumped by flow reversal to the packed microcolumn for PCB uptake at 0.5 mL min^{-1}. This procedure is repeated threefold, whereby a total amount of 12 mL of sample is loaded. The air plug is finally delivered to the column for drying of the beads prior to initiate the elution step.

Step 3: Elution. In order to prevent the introduction of aqueous segments into the GC transfer line the eluent zone was followed

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by an air plug. On that account, an amount of 250 μL of air is first aspirated into HC, whereupon a minute, well-defined organic eluent volume (80 μL) is aspirated. The eluent zone is divided in two parts, each being halted into the column for 15 s for effective stripping out of PCBs. It should be stressed that both eluate and transfer lines were filled with air prior to elute the retained analytes.

Step 4: Transportation of Eluate. Once analyte elution is completed, the rotary injection valve is automatically activated to the load position and the eluate zone is transported to the valve loop followed by the air segment stored in the HC. The injection valve is next switched to the inject position, and the eluate is forthwith delivered to the GC by a gentle stream of air provided by S1. The GC is at this moment activated, and the temperature programs of the injector and column oven are initiated. The GC separation is actually synchronized with the BI-LOV sorptive procedure, implying that a sample is analyzed while the ensuing one is being processed in the flow system. Total sample preparation time in LOV and transportation of eluate into PTV takes ca. 45 min, thus closely matching the time frame for both the chromatographic run and column/injector re-equilibration to the initial conditions, which amounted to 50 min.

Step 5: Bead Discarding and System Conditioning. Since the polymeric beads are extremely easy to handle within the integrated microconduits, the packed column reactor is facilely backflushed after being moistened with methanol and delivered to waste (port 2) with carrier solution. Further, the transfer line to GC is cleansed with an air-segmented volume of ethyl acetate, which is dispensed to waste via the additional solenoid valve integrated into the manifold. Hence, the flow system is ready to initiate a new analysis cycle with a fresh portion of beads, thus eliminating any possibility of cross-contamination between consecutive runs.

RESULTS AND DISCUSSION

Configuration of the MSFI-LOV−GC System. A crucial aspect in online hyphenation of SPE to GC is to possess means for reliable delivery of eluate into the injector. Thus, a vast number of researchers have opted for exploitation of syringe pumps as liquid drivers for accurate handling of minute volumes of eluent.[6,8,10,39−41] As a consequence of the stringent demands of PTV-GC as to the maximum solvent volume to be accommodated into the injector in a single step to prevent column flooding (<100−200 μL), heart-cut injections have been proposed[8] despite the partial loss of the preconcentration capabilities gained during analyte sorption. Most common is the introduction of the entire volume of eluate into the GC.[6,8,10,39−41] Yet, the use of a solvent stream as carrier leads to increased dispersion of eluate in the transfer line with the consequent undue dispersion of eluted analytes. To overcome this drawback, we selected an air-sandwiched type elution as earlier described in BI-LOV systems coupled to discontinuous operating detectors[18,23,24] for accurate injection of discrete volumes of eluate. Yet, direct air segmentation into the transfer line was proven inappropriate as a consequence of the buildup of back-pressure by the capillary tubing tailored to the PTV injector which compressed the air segments and rendered irreproducible solvent transfer. Thus, an ancillary rotary valve furnished with an eluate loop was in this work implemented into the flow network to feed the injector with the eluted analytes via a pressurized air stream provided by S1 (see Figure 1).

Several PTV conditions, namely, purge (vent) time, purge temperature, and purge flow, were explored for appropriate performance of the injector. Among them, the former was proven to be the most relevant parameter. Early vent end times tended to yield irreproducible results due to incomplete solvent elimination and lack of instant inlet pressure equilibration from the (negative) pulse of 1 psi at the injection to the working pressure (∼15 psi) required for maintaining a constant GC flow of 1.3 mL min^{-1}. This problem was solved by setting the vent time to 1.5 min while affixing the injector temperature and split ratio to 70 °C and 60, respectively. No losses of the most volatile PCBs were observed under the vent time selected for solvent evaporation.

Notwithstanding the fact that the use of sorbent-packed liners has been proposed for uptake of the eluate to prevent early introduction of analytes into the GC capillary column,[10] the liners were, however, in this work simply packed with glass wool. The latter is less retentive than sorbent materials, e.g., carbofrit, which avoids eventual carryover effects in the injector while assuring efficient collection of eluate. It should be stressed that a good discrimination between PCBs and solvent is accomplished in the venting step as a consequence of the low volatility of the target compounds.

Selection of BI-SPE Sorbent and Eluent for LOV Analysis. Four different reversed-phase sorbent materials, namely, Oasis HLB, Lichrolut EN, Bond Elut Plexa, and spherical Upti-Clean C18, were evaluated as regards their ability for retention and elution of the target analytes, as well as their ease for handling in LOV in a renewable mode. They offer different chemical functionalities, and to our best knowledge, merely copolymeric *N*-vinylpyrrolidone−divinylbenzene (Oasis HLB) beads have been successfully tested in a BI-LOV operation.[36,42] In fact, automated manipulation in aqueous media of hydrophobic and high-density sorbents (e.g., C18 covalently modified silica gel or PTFE) in LOV had been proven rather cumbersome to date because of their ease of settling down within the flow conduits.[18,43] The sorption/elution performance of the overall sorbents was initially tested in combination with three eluents of different polarity (viz., isooctane, toluene, and ethyl acetate) by retention of a given amount of the suite of PCBs followed by consecutive elution in three to four fractions of 80 μL each. Isooctane was proven not suitable for quantitative stripping out of any of the selected PCBs; even with a total solvent volume of 320 μL (4 × 80 μL), the amount of either of the analytes eluted was lower than 70% (data not given). On the other hand, ethyl acetate featured enhanced elution strength, with more than 80% of the PCBs being eluted in a single 80 μL

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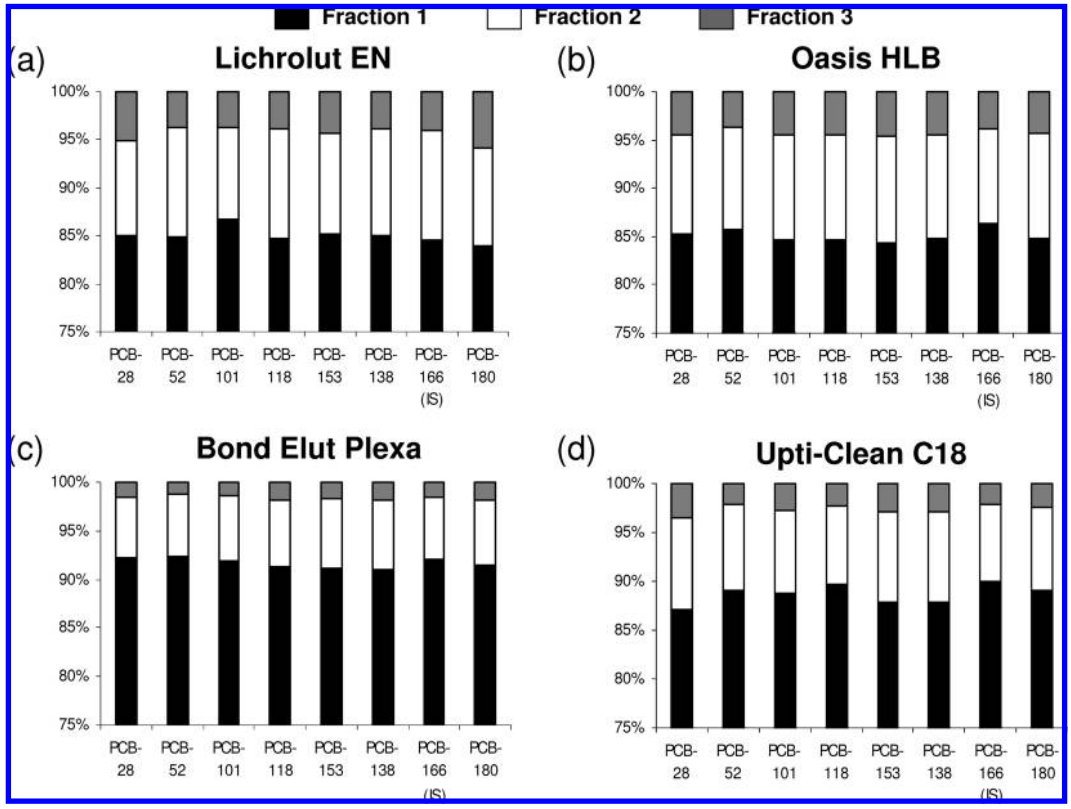

Figure 2. Normalized elution profile of different sorbents with three 80 μL fractions of ethyl acetate in BI-LOV ($n = 3$): (a) Lichrolut EN, (b) Oasis HLB, (c) Bond Elut Plexa, and (d) Upti-Clean C18. Beads were loaded with 1.0 mL of standard containing 50% (v/v) methanol spiked at the 5 μg L^{-1} level. N.B.: *y*-axis scale 75−100%.

fraction (Figure 2). This eluent was particularly advantageous in combination with the copolymeric Bond Elut Plexa sorbent (Figure 2c), where an elution efficiency >90% was achieved in the first fraction. The improved performance of ethyl acetate might be attributed to its partial solubility with remains of water left onto the beads due to incomplete air-drying of the sorbent under moderate/low pressure (see the Materials and Methods section). In fact, ethyl acetate has been recommended by Hyötyläinen and Riekkola in several reviews dealing with online SPE−GC coupling, because of the formation of a volatile azeotrope with water.[3,4] Toluene provided initially similar results as those attained with ethyl acetate, but its usage resulted into the deterioration (partial dissolution) of the Ultem material of the LOV after a few cycles and was therefore discarded.

As regards their physical manipulation in LOV as renewable sorptive surfaces, all the four sorbents could be handled within the microfabricated conduits of the integrated LOV platform in amounts varying from 2.5 (Oasis HLB) to 4 mg (spherical C18) per column in the conditions given under the Materials and Methods section. However, Lichrolut EN and Upti-Clean C18 sorbents were difficult to renew whenever sample volumes ≥10 mL were preconcentrated. This observation was attributed to the nonspherical shape of Lichrolut EN beads and the hydrophobic nature of the C18 material. On the other hand, the lipophilic/hydrophilic copolymeric Oasis HLB sorbent had already shown its good performance in LOV because of its good water wettability.[36,42] This holds true for Bond Elut Plexa beads, which consist of a hydrophobic polystyrene−divinylbenzene copolymer core, covered by a hydrophilic hydroxylated surface. In terms of

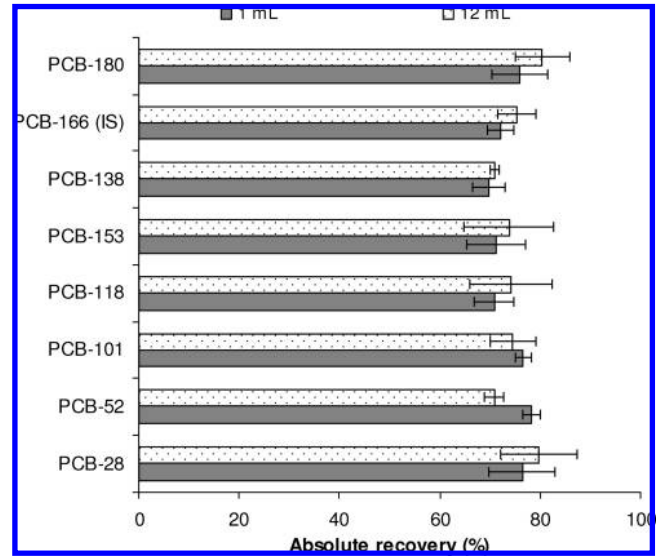

Figure 3. Comparative evaluation of absolute recoveries in BI-LOV as obtained by loading on Bond Elut Plexa either 1.0 or 12 mL of standard solutions spiked with 2 ng of each PCB congener (including the IS) containing 50% (v/v) methanol ($n = 3$).

PCBs retention capacity, all sorbents performed equally well, without appreciable analyte breakthrough when increasing the loading volume from 1 to 12 mL, in spite of the small amount of sorbent packed and the progressive loading of the SPE column with the methanol in the standards, as exemplary illustrated in Figure 3 for Bond Elut Plexa beads.

Table 1. Analytical Performance of the Online BI-LOV−GC Method for Determination of Trace Level Concentrations of PCBs

			recovery (% RSD)—sample no. 1[c]		recovery (% RSD)—sample no. 2[d]	
	linearity (r^2)[a]	LOQ (ng L^{-1})[b]	10 ng L^{-1}	50 ng L^{-1}	10 ng L^{-1}	50 ng L^{-1}
PCB-28	0.9993	4.1	107.4(12.8)	103.1(6.8)	91.3(9.3)	92.0(4.6)
PCB-52	0.9992	6.1	99.7(5.7)	87.9(4.4)	89.5(10.2)	94.7(5.0)
PCB-101	0.9999	2.0	99.1(5.2)	90.7(5.9)	81.4(9.1)	90.0(4.8)
PCB-118	0.9992	1.2	100.9(7.6)	90.3(5.4)	87.6(9.4)	97.0(4.3)
PCB-153	0.9992	1.4	103.1(4.2)	88.7(4.2)	86.4(2.0)	91.7(2.3)
PCB-138	0.9989	1.0	116.2(11.5)	100.2(8.8)	98.4(8.5)	102.2(7.1)
PCB-180	0.9977	0.5	112.5(7.8)	93.9(4.9)	90.7(7.9)	99.8(1.2)

[a] Six-point calibration (2, 5, 10, 20, 50, and 100 ng L^{-1}). [b] Calculated as the signal for which the signal-to-noise ratio is 10 using a spiked landfill leachate sample at the 10 ng L^{-1} level. [c] Sample no. 1: landfill leachate ($n = 4$). [d] Sample no. 2: bulky and electronic waste disposal leachate ($n = 4$);0.1% (w/v) disodium EDTA and 0.75% (v/v) concentrated NH_3 were added to this sample in order to prevent precipitation of metal ions.

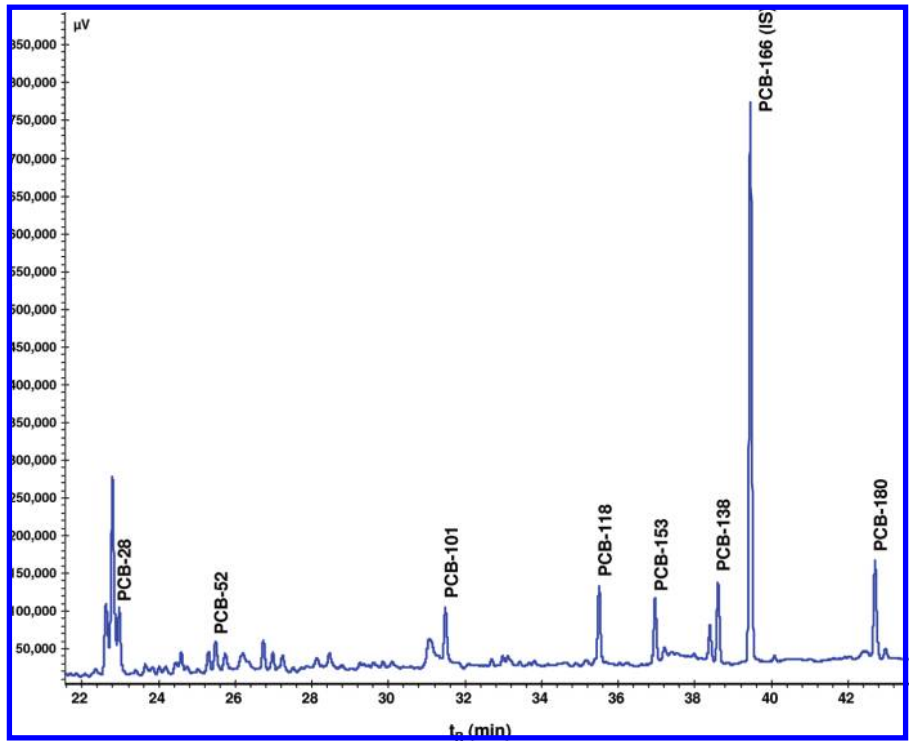

Figure 4. Chromatogram of a landfill leachate sample (sample no. 1) spiked at the 10 ng L^{-1} level of each PCB congener and 50 ng L^{-1} of IS (PCB-166).

The combination of Bond Elut Plexa beads and elution with a single plug of 80 μL of ethyl acetate was selected as a compromised approach for sensitive and expeditious BI-LOV−GC analysis. Absolute recoveries, as compared to manually injected standards, were >70% (Figure 3), and fast mass transfer of PCBs from hydroalcoholic media is warranted as a consequence of the hydrophilic surface of the beads. Analyte recoveries could be improved by increasing the elution volume, yet this would entail further optimization of the PTV parameters and more complex operational protocols. It should be borne in mind that nonquantitative recovery of analytes in BI-LOV−GC procedures should not be in detrimental of the method's trueness and precision, because of the high reproducibility in fluid handling in automated flow systems along with the fact that a surrogate IS is added to both samples and standard solutions, calibration is performed under identical experimental conditions than sample analysis, and the entire SPE column is withdrawn and replaced by fresh beads after each single assay to eliminate potential analyte carryover sources.

Analytical Performance of the BI-LOV−GC System. The analytical performance of the proposed procedure using Bond Elut Plexa beads in a renewable fashion is summarized in Table 1 including linearity, quantification limits (LOQ), and recoveries at the 10 and 50 ng L^{-1} levels. The online BI-LOV−GC method features an excellent linearity, with correlation coefficients >0.9977 for the entire suite of PCB congeners, taking into account that the SPE column is merely utilized in a single analytical run. The LOQ values, calculated at a peak-to-peak signal-to-noise ratio (S/N) of 10 for a spiked landfill leachate sample at the 10 ng L^{-1} level (Figure 4), ranged from 0.5 to 6.1 ng L^{-1}, thereby sufficing for determination of PCBs in leachates at environmentally relevant concentrations. In fact, the enrichment factors under the optimized experimental conditions were

Table 2. Comparison of Expected and Found Concentrations of PCB Congeners in a Landfill Leachate Spiked with Aroclor 1260 at the 500 ng L^{-1} Level

	expected concn (ng L^{-1})[a]		found concn (ng L^{-1})[b]		Student t test	
	mean	SD	mean	SD	calcd t	statistically different?[c]
PCB-28	2.1	0.2	<LOQ			
PCB-52	2.39	0.09	<LOQ			
PCB-101	24.0	1.5	22.6	0.9	1.37	no
PCB-118	3.7	0.6	3.2	0.5	1.21	no
PCB-153	75.5	3.4	76.7	1.7	0.55	no
PCB-138	57.0	3.3	59.8	1.6	1.27	no
PCB-180	59.6	2.9	62.2	3.5	0.97	no

[a] Calculated from the concentration of each PCB in the Aroclor spike as determined by GC ($n = 3$). [b] $n = 3$. [c] 95% confidence level; $t_{critical} = 2.78$.

>52. Further, the method's selectivity and LOQs could be readily improved by coupling the miniaturized BI-LOV system to gas chromatography/high-resolution mass spectrometry (GC/HRMS) whenever needed.

Trueness and precision of the method were studied using two real-life samples, namely, leachates from an urban solid waste landfill (sample no. 1) and an electronic equipment disposal site (sample no. 2), which were analyzed and proven not to contain detectable amounts of PCBs. Both samples were spiked at the 10 and 50 ng L^{-1} levels, and the analytical recoveries and relative standard deviation were thus calculated. As compiled in Table 1, relative recoveries varied from 81% to 116% for both samples, and relative standard deviations were found to range from 2.0% to 13% and 1.2% to 8.8%, for 10 and 50 ng L^{-1} level spikes, respectively. It should be noted that the proposed analytical method features under the selected experimental conditions better accuracy and reproducibility than those recommended by the EPA method 1668a for PCBs determination, where the quality control criterion establishes that the precision and recovery in standard solutions should be better than 40% RSD and within the range from 60% to 140%, respectively.[44]

Because none of the analyzed samples contained PCBs, another landfill leachate (sample no. 3) was spiked with Aroclor 1260, one of the most common PCB mixture still in use in some old electrical transformers and capacitors, in order to analyze a more environmentally realistic sample in terms of matrix complexity and congener's profile and, hence, evaluate the reliability and ruggedness of the hyphenated LOV−GC system. The Aroclor mixture was first characterized by direct injection in the GC (see first two columns in Table 2) and then spiked into the leachate sample. The spike level was set to 500 ng L^{-1}, as total Aroclor, because this is the maximum concentration level permitted by the U.S. EPA National Primary Drinking Water Standards.[45] It should be here highlighted the fact that the landfill and dump leachate contamination of water sources might pose serious risks to public health. A chromatogram of the spiked landfill leachate is presented in Figure 5, and experimental results are compiled in Table 2. A t test of comparison of means[46] confirmed the inexistence of significant differences between the expected and found concentrations for the overall analytes at the 0.05 significance level.

The comparison of the BI-LOV−GC approach with previous online SPE−GC methods reported in the literature reveals that, on one hand, the recoveries and RSD values obtained in this work are similar or better than those of commercial robotic Prospekt-type instruments or in-house systems, where the sorbent is usually reused and synthetic aqueous matrixes, most often ultrapure water, in lieu of real-life samples are employed for method validation.[6−8,10] On the other hand, the expenses in BI-LOV in

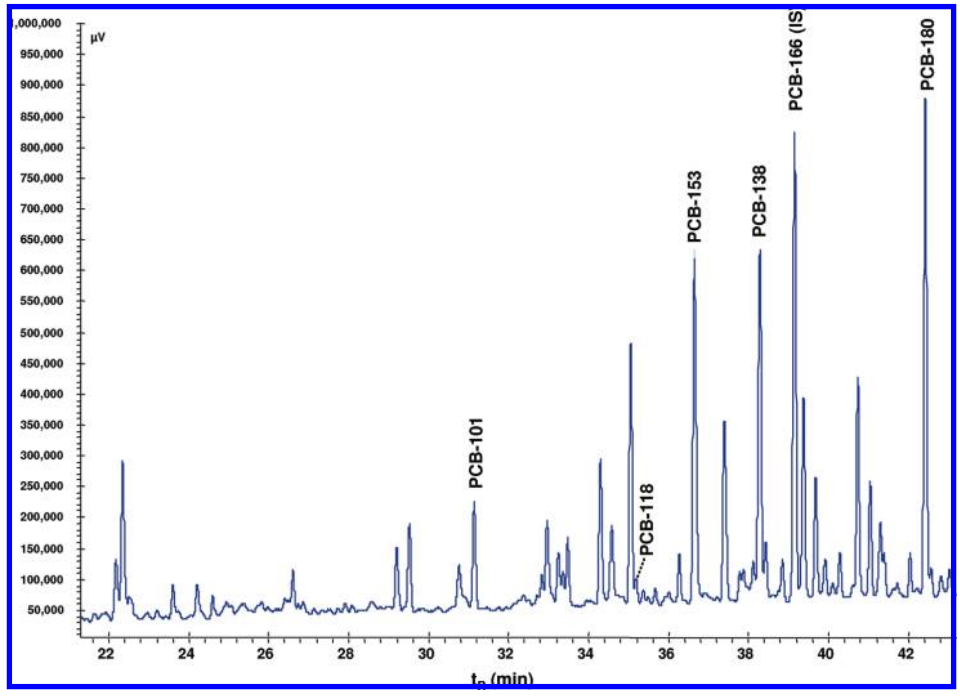

Figure 5. Chromatogram of a solid waste leachate sample (sample no. 3) spiked with 500 ng L^{-1} of Aroclor 1260 and 50 ng L^{-1} of IS (PCB-166).

Table 3. Comparative Evaluation of Sorbent Costs for Analytical Methods Involving SPE

extraction fashion	typical format	approximate cost per 100 analyses (euro)
off-line	syringe barrel/disk (C18 or polymeric)	150−400[a]
online Spark Holland systems	Prospekt or Prospekt-2 cartridges	300−500[a,b]
online reusable precolumn	SPE precolumn (C18 or polymeric; 2.1−4.6 mm × 20 mm)	150−400[a,c]
online BI-LOV−GC (this work)	LOV microcolumn (3 mg Plexa)	∼6

[a] Estimated range depending on sorbent material employed (C18, polymeric, etc.). [b] Cartridge is renewed after every sample/standard analysis. [c] Assuming the reutilization of a single precolumn for 100 analyses.

terms of sorbent material are 25-fold lower than those of off-line and online SPE counterparts (see Table 3). In fact, some researchers using robotized systems decided to reuse cartridges to reduce costs,[6] in spite of risks of sample cross-contamination.

Finally, it is also worth mentioning that the GC equipment operated for more than 200 standards and samples analyses without column performance deterioration. This can be partly attributed to the restricted access material (RAM)-like nature of Bond Elut Plexa beads that prevents nonvolatile macromolecules from being retained and further eluted and transferred to the GC injection port and column head.

CONCLUSIONS

Online hyphenation of BI-LOV with GC has been in this paper for the first time demonstrated. This new approach fostered the implementation of SPE and determination of PCBs from landfill leachate samples in a fully automated fashion with the advantages over traditional online SPE−GC methods of simplicity, versatility, and inexpensiveness as compared to robotic systems (e.g., Prospekt, Prospekt-2) and negligible cross-contaminations as a consequence of the renewal of the sorbent material in each analysis cycle. The automated BI-LOV−GC method is highly sensitive (LOQs between 0.5 and 6.1 ng L^{-1} for 6 mL sample (12 mL after 1:1 dilution with MeOH)), accurate (recovery percentages >81%), and reproducible with RSD values lower than 13% for real leachate samples. Indeed, this first work opens new horizons for hyphenation of LOV microfluidic analysis with GC, including the potential interface of BI-LOV with GC/(HR)MS for water and beverages analysis or the coupling of flow-through dynamic fractionation schemes[25,47] to GC via the LOV platform for availability studies of organic pollutants in environmental solid substrates.

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(45) U.S. EPA. National Primary Drinking Water Standards. http://www.epa.gov/safewater, 2003.
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NOTE ADDED AFTER ASAP PUBLICATION

This paper was published ASAP on May 13, 2009 with an error in Table 2. The corrected version was published ASAP on May 22, 2009.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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CHAPTER 10

CONCLUSIONS

Chapter 10

CONCLUSIONS AND FUTURE WORK

Novel automatic sample preparation methods resorting to the various generations of flow analysis have been in this dissertation proposed and validated for extraction, dynamic fractionation, preconcentration, separation and determination of trace level concentrations of inorganic and organic contaminants in environmental samples of varied matrix complexity including environmental waters, waste leachate landfills, agriculture soils, coal fly ash, and biomass fuels.

For assays of inorganic trace constituents in solid substrates, a rugged, fully automated multisyringe-based stirred-flow chamber extraction system has been characterized and validated for simultaneous fractionation analysis of three solid wastes as expeditious alternative to flow-through microcolumn methods. This method has been proven suitable for expedient assessment of the size of the most potentially mobile pools of hazardous elements because it guarantees complete leaching of targeted phases thereby mimicking leaching under worst case conditions as endorsed in ISO norms. Further, it has potential for routine analysis aimed at ascertaining the eventual end use of solid waste for civil engineering works and soil amendment. Most importantly, the extraction chamber admits larger sample amounts (≤1.0 g) as compared to a mere few micrograms (usually ≤50 mg) in flow injection/sequential injection microcolumn approaches with no effect of leachant flow rate up to 6.0 mL min^{-1}. Therefore, the devised flow-through extractor is particularly suited for automatic fractionation of heterogeneous solid samples, such as industrial solid byproducts.

Another relevant asset of the proposed flow-through dynamic fractionation method is that within the investigated intervals neither the solid to liquid ratio nor the extraction time is a critical variable for evaluation of maximum metal bioaccessibility (demonstrated also by application of experimental designs). This was corroborated by critical comparison of two novel automatic fractionation schemes, the so-called SI-MCE and SI-SFCE, for determination

of overall mobilizable pools of trace elements in NIST 1633b fly ash exploiting the standard BCR leaching test. The significant decrease of analysis times in both BCR fractionation methods, namely, 3.3 h as compared with 48 h for batchwise BCR is also worth mentioning We conclude that the extraction conditions in both SI-MCE and SI-SFCE, e.g., sample amount and solid-to-liquid ratio, could be readily modified attending to the needs of the particular assay (e.g., sample availability and representativeness) without statistically significant changes on BCR metal percentage leachability, provided that exhaustive extraction is ensured and appropriate sample containers are designed.

The application of dynamic fractionation methods capitalized on SI-SFCE as a front end to ICP-OES afforded fast assessment of readily mobilisable forms of alkali and alkaline-earth elements (e.g., K, Ca, Na and Mg) in biomass resources (pine twigs and bark) to provide rapid results to industry to elucidate potential fireside problems when firing the woody biofuels. The automated method offers excellent intermediate precision, improved laboratory safety as compared to batchwise counterparts, reduced risks of sample contamination and minimal operational maintenance. Besides, the overall sequential extraction scheme is accomplished in less than 3 hours in lieu of 7 days whenever undertaken in a manual fashion. Furthermore, combination of dynamic fractionation with SEM-EDXA and XRD for elucidation of metal-biomass/ash associations made it a more useful tool for combustion technology and investigation of the actual selectivity of extraction reagents.

For assays of organic trace constituents in environmental samples, automatic sample preparation methods exploiting sorbent-based extraction techniques has been characterized and validated for preconcentration, purification, and determination of trace level concentrations of organic persistent compounds in a vast number of troublesome matrices prior to chromatographic separations. A stirred-flow microcolumn configuration for handling of dispersed carbon nanomaterials (cup-stacked carbon nanotubes, CNT) as sorptive surfaces has been proven appropriate for μSPE and cleanup of triazine herbicides of differing polarity in both environmental waters and soil extracts with minimum nanoparticle agglomeration and negligible pressure drop. The beauty of the devised assembly is that nanoparticles are to be used as-purchased with no need for cumbersome immobilization procedures to afford composite core-shell nanoparticles or decorated membranes. Not the least, multistage μSPE procedures including sorbent regeneration have been fully automated by resorting to programmable flow inherent to sequential injection analysis. The versatile at-line interface to

LC fosters independent optimization of the CNT-based sample processing regardless of the eluent volume for quantitative stripping of preconcentrated species. The hyphenated analyzer provides sufficient sensitivity and reliability for determination of triazine herbicides at concentration levels below those specified by current legislations for human water consumption and surface waters. This work results in significant progress toward the development of miniaturized and simplified sample handling methodologies with green chemical credentials because of the use of minute amounts of nanomaterials and organic solvents contrarily to manual SPE cartridges.

Due to the low concentration levels of triazines and the effect of interfering matrix ingredients in soil extracts, multimodal SPE involving two kinds of beads (reversed-phase copolymeric and MIP sorbents) have been employed aimed at the concomitant enhancement of selectivity and retention efficiency of target compounds in the sorptive extraction procedure. In-line *μ*SPE with merely *N*-vinylpyrrolidone-divinylbenzene is however recommended for environmental water assays so as to simplify the system without compromising the selectivity. After appropriate scrutiny of the various parameters governing the performance of the system, the flow analyzer provides sufficient sensitivity and reliability for long-term assays at trace concentration levels of herbicides. The flow analyzer hyphenated to LC has proven suitable to not merely handle both aqueous and organic solutions in a single automatic setup encompassing in-line sample pretreatment but effectively solve LC band-broadening effects via in-line dilution of the SPE eluate.

In this thesis, Bead Injection (BI, renewable μSPE) using mixed-mode sorbent materials has been for the first time allied to GC for on-line μSPE in a mesofluidic Lab-on-a-Valve (LOV) format for automated determination of trace level concentrations of polychlorinated pollutants. This new approach fostered the determination of PCBs in landfill leachate samples in a fully automated fashion with the advantages over traditional online SPE-GC methods of simplicity, versatility, and cost-effectiveness as compared to commercially available robotic systems (e.g., Prospekt, Prospekt-2 from Spark Holland) and negligible cross-contaminations as a consequence of the renewal of the sorbent material in each analysis cycle. The automated BI-LOV-GC method is highly sensitive (LOQs between 0.5 and 6.1 ng L^{-1}), accurate (recovery percentages >81%), and reproducible with RSD values lower than 13% for real leachate samples. Indeed, this work opens up new horizons for hyphenation of LOV

microfluidic analysis with GC, including the potential interface of BI-LOV with GC-MS for water and beverages analysis or the coupling of flow-through dynamic fractionation schemes to GC *via* the LOV platform for bioaccessibility tests of organic pollutants in environmental solid substrates and foodstuffs.

Future work along the lines of the research conducted in the framework of the PhD thesis is currently underway for:

- Development of dynamic bioaccessibility and speciation methods for metalloid compounds in environmental samples.
- On-line handling of solid extracts via membrane separation approaches, e.g., gas diffusion, (micro)dialysis and pervaporation.
- Development of automated *in-vitro* oral bioaccessibility methods for assessment of available pools of pesticides in foodstuff after ingestion.
- Broaden the applicability scope of automatic chemical fractionation methods for advanced characterization of biomass resources other than twigs and bark, including waste derived fuels (e.g., sewage sludge and agriculture wastes, such as straw), and troublesome fuel blends as well.
- Expand the stirred-flow microcolumn configuration and LOV platform for handling of metal/carbon nanoparticles or composites within μSPE procedures for assays of priority pollutants in environmental matrixes and for bioassays of endogenous organic species and xenobiotics as well.

APPENDIX

CURRICULUM VITAE

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PERSONAL INFORMATION

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EDUCATION

Bachelor of Science in Chemistry (Honor), **1997-2000**
Department of Chemistry, Faculty of Science, Maejo University, Chiangmai, Thailand
Bachelor project: *Characterization of medicine table by High performance Liquid Chromatography: Clinical Applications*

Master of Science in Applied Analytical and Inorganic Chemistry, **2001-2004**
Department of Chemistry, Faculty of Science, Mahidol University, Bangkok, Thailand
Thesis: *Chemometric approach to acid-base properties of humic acids and their interactions with calcium cation*

Ph.D. Student in Analytical Chemistry, **2004-2007**
Department of Chemistry, Faculty of Science, Mahidol University, Bangkok, Thailand

Master of Science and Chemical Technology, 2008
Department of Chemistry, University of the Balearic Islands, Illes Balears, Spain

Ph.D. Student in Science and Chemical Technology, 2009-2012
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RESEARCH AND TEACHING EXPERIENCE

1 Staff of pharmaceutical analysis via HPLC Project, **1999**, Maejo University, Chiangmai, Thailand.

2 Assistant Researcher: Trace analysis of raw materials for Feedstuff, Charoen Pokaphand Foods Public Co Ltd**.**, **2000**, Thailand.

3 Assistant Researcher: Size-based trace metals analysis in sedimentation**,** Ministry of Science and Technology, **2001-2003**, Bangkok, Thailand.

4 Teaching Assistant, Analytical Instrument Laboratory Course, Mahidol University, **June 2003 - March 2004**, Bangkok, Thailand.

5 Assistant Researcher: Hydrogen production from ethanol reformer process for Solid Oxide Fuel Cell, National Metal and Materials Technology Center (MTEC), **2005-2006**, Bangkok, Thailand.

6 Teaching Assistant, General Chemistry Laboratory Course, Mahidol University, **June 2007 - September 2007**, Bangkok, Thailand

RESEARCH STAY AND VISIT

1 Physical-Chemistry department, Faculty of Pharmacy, University of Porto, **1 May - 30 July 2009**, Portugal to conduct research in the topic of *Nano-structured adsorbents for extraction and preconcentration of priority organic pollutants*.

2 Physical-Chemistry department, Faculty of Pharmacy, University of Porto, Portugal, **24 February - 3 March 2010** in order to fulfil the research collaboration agreement ″HP2008-0045″ (Acción Integrada) funded by the Spanish Government.

3 Institute of Food Analysis and Research, Department of Analytical Chemistry, University of Santiago de Compostela, Spain, **20 May - 31 July 2010** to conduct research in the topic "*On-line sorptive microextraction coupled to PTV-GC-MS/MS for fully automated determination of organochlorine pesticides in surface waters and waste*

waters".

4 Åbo Akademi University, Process Chemical Centre Laboratory of Analytical Chemistry, Åbo-Turku, Finland, **5 August - 22 October 2010** according to Johan Gadolin Fellowship in the topic of "*Development of a novel in-line dynamic fractionation method coupled to inductively coupled plasma-atomic emission spectroscopy and inductively coupled plasma-mass spectrometry for bioaccessibility tests of ash-forming elements in biomass fuels."*

5 School of Chemistry, The University of Melbourne, Victoria, Australia, **1 June - 31 August 2011** in the topic of "*Development of flow-based analytical methods for arsenic speciation and separation in troublesome environmental samples using pervaporation."*

RESEARCH GRANTS

1 Charoen Pokaphand Group, **June 1999 - March 2000**, Bangkok, Thailand

2 Postgraduate Education and Research Program in Chemistry (PERCH) Fellowship**, June 2001 - March 2002**, Mahidol University, Faculty of Science, Bangkok, Thailand.

3 Young Scientist Scholarship, **June 2004 - June 2007**, Faculty of Science, Mahidol University, Bangkok, Thailand.

4 Fellowship from "I Call for Strengthen the Educational Systems of Developing Countries: Research Stays" ,**October 2007 - November 2007**, at University of the Balearic Islands, Spain.

5 Partner in the research Project at UIB: "Development of Analytical Methods for Determination of Priority Pollutants in Solid Incineration Wastes", **December 2007 - September 2008**, University of the Balearic Islands, Spain (Project PROGECIB 1A) and Special Action both from Conselleria d'Economia, Hisenda i Innovació of the Government of the Balearic Islands.

6 Ph.D. grant from the Conselleria d'Educació, Cultura i Universitats, Direcció General d´Universitats, Recerca i Transferència del Coneixement from the Government of the Balearic Islands, Spain, for financial support through European Social Fund (ESF), **October 2008 - September 2012**.

7 Johan Gadolin Fellowship at Åbo Akademi Process Chemistry Centre Laboratory of Analytical Chemistry, **August 2010 to October 2010**, Åbo Akademi University, Process Analytical Chemistry Centre, Turku, Finland.

PROJECTS

1. Partner in the research Project at UIB and University of Porto: "Characterization and evaluation of the use of nanostructured solid phases for automatic extraction and preconcentration of pollutants", Ministerio de Ciencia e Innovación-Acción integrada con Portugal (Project reference number: HP2008-0045), **2009-2010.**

2. Partner in the research Project (at UIB): "*¿Está perjudicando la sobreexplotación agrícola del Norte de Tailandia la calidad del suelo y la de los recursos hídricos del país?*", Consejería de Asuntos Sociales, Promoción e Inmigración del Gobierno de las Illes Balears en colaboración con la Universidad de las Illes Balears, **November 2009 - December 2010.**

3. Partner in the research Project (at UIB): "*Impulso a la producción de uva de mesa y vinificación como mercado emergente en el Norte de Thailandia"*, COOD-Convocatòria d'Ajuts de Cooperació al Desenvolupament: Projectes de Cooperació al desenvolupament. University of the Balearic Islands and Government of the Balearic Islands, **2009-2010.**

4. Partner in the research Project (at UIB): "*Métodos analiticos automaticos basados en fraccionamiento dinamico para determinar la biodisponibilidad de contaminantes ambientales en suelos, sedimentos y residuos solidos"*, granted by the Ministerio de Ciencia e Innovación (MICINN) - Plan nacional de Investigación científica, desarrollo e innovación tecnológica, Project reference number: CTM 2010-17214, **2011**.

PUBLICATIONS

1. **Combination of Electrochemical Impedance Spectroscopy and Cyclic Voltammetry to Investigate Oxygen Ion Transport in YSZ Electrolyte of SOFC**, Warunya Boonjob, Nitinai Punbusayakul, Ron Beckett, Manop Masomtub, Kitsadate Wongtidad, Sumittra Charojrochkul, and Waret Veerasai, Electro Chemical Society Transaction. **7**, (1) 1293-1295 (2007).

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