Development of Methodologies for Determination of Trace-level Concentrations of Elements by Atomic Spectrometry via On-line Pretreatment Procedures Exploiting Sequential Injection (SI) Lab-on-Valve (LOV) Schemes

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Development of Methodologies for Determination of Trace-level Concentrations of Elements by Atomic Spectrometry via On-line Pretreatment Procedures Exploiting Sequential Injection (SI) Lab-on-Valve (LOV) Schemes

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Ph.D. Thesis

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Preface

This thesis represents a part of the requirements for obtaining the Ph.D. degree at the Technical University of Denmark (DTU). The study was financed by a Ph.D. stipend granted by DTU and was carried out during the years 2003-2006 mainly at the Department of Chemistry at DTU, but also in part at the Department of Chemistry at the University of the Balearic Islands, Spain (three months external research).

This thesis is based on the following 8 publications in peer-reviewed journals, which form integrate parts of the thesis, describing the development of methodologies for determination of trace levels of metal and metalloid elements in environmental samples via on-line pretreatment procedures exploiting sequential injection (SI) lab-on-valve (LOV) schemes.

I. Xiangbao Long, Roongrat Chomchoei, Piotr Gała, Elo Harald Hansen, *Evaluation of a novel PTFE material for use as a means for separation and preconcentration of trace levels of metal ions in sequential injection (SI) and sequential injection lab-on-valve (SI-LOV) systems. Determination of cadmium(II) with detection by electrothermal atomic absorption spectrometry (ETAAS),* Anal. Chim. Acta, 2004, **523**, 279-286.

II. Xiangbao Long, Elo Harald Hansen, Manuel Miró, *Determination of trace metal ions via on-line separation and preconcentration by means of chelating Sepharose beads in a sequential injection lab-on-valve (SI-LOV) system coupled to electrothermal atomic absorption spectrometric detection,* Talanta, 2005, **66**, 1326-1332.

III. Xiangbao Long, Manuel Miró, Elo Harald Hansen, *An automatic micro-sequential injection bead injection lab-on-valve (μSI-BI-LOV) assembly for speciation analysis of ultra trace levels of Cr(III) and Cr(VI) incorporating on-line chemical reduction and employing detection by electrothermal atomic absorption spectrometry (ETAAS),* J. Anal. At. Spectrom., 2005, **20**, 1203-1211.

IV. Xiangbao Long, Manuel Miró, Elo Harald Hansen, *Universal Approach for Selective Trace Metal Determinations via Sequential Injection-Bead Injection-Lab-on-Valve (SI-BI-LOV) Using Renewable Hydrophobic Bead Surfaces as Reagent Carriers,* Anal. Chem., 2005, **77**, 6032-6040.

V. Xiangbao Long, Manuel Miró, Elo Harald Hansen, *On-line dynamic*

extraction and automated determination of readily bioavailable hexavalent chromium in solid substrates using micro-sequential injection bead-injection lab-on-valve hyphenated with electrothermal atomic absorption spectrometry, Analyst, 2006, **131**, 132-140.

VI. Elo Harald Hansen, Manuel Miró, Xiangbao Long, Roongrat Petersen, *Recent developments in automated determinations of trace level concentrations of elements and in on-line fractionations schemes exploiting the micro-sequential injection-lab-on-valve approach*, Anal. Letters, 2006, **39**, 1243-1259.

VII. Xiangbao Long, Manuel Miró, Rikard Jensen, Elo Harald Hansen, *Highly selective micro-sequential injection lab-on-valve (μSI-LOV) method for determination of ultra trace concentrations of nickel in saline matrices using detection by electrothermal atomic absorption spectrometry*, Anal. Bioanal. Chem., 2006, **386**, 739-748.

VIII. Xiangbao Long, Manuel Miró, Elo Harald Hansen, José Manuel Estela, Víctor Cerdà, *Hyphenating multisyringe flow injection lab-on-valve analysis with atomic fluorescence spectrometry for on-line bead-injection preconcentration and determination of trace levels of hydride-forming elements in environmental samples*, Anal. Chem., In press.

The author has also been co-author on the following publication, which is not included in the thesis:

IX. Elo Harald Hansen, Roongrat Chomchoei, Xiangbao Long, *Three Generations of Flow Injection Analysis* (in Danish), Dansk Kemi, 2004, **85**(10), 58-63

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Xiangbao Long

Abstract

Sample pretreatment is often a necessary step in analysis of real samples which contain trace/ultra-trace level concentrations of the measurand - especially when the detector used does not exhibit extremely high sensitivity - and comprise complex matrices that can interfere in the analysis. Among the many pretreatment techniques available, the use of the third generation of flow injection, the so-called sequential injection lab-on-valve, is probably the most promising approach. The present Ph.D. thesis is focused on exploitation of the versatility of the sequential injection lab-on-valve (SI-LOV) system for the development of robust on-line automatic SI-LOV pretreatment procedures employing solid phase extraction (SPE) for separation and preconcentration of trace elements in environmental samples coupled with various atomic spectrometric detection techniques. Taking advantage of the precise and reproducible timing and versatility of the SI-LOV system and that the solid-phase bead materials used can be renewed whenever called for, special attention is placed on the intelligent exploitation of the interplay between the thermodynamics and the kinetics of reactions involved, that is, executing kinetic discrimination schemes, which in turn has resulted in the development of a number of novel concepts as illustrated by the procedures detailed in the scientific publications which have arisen from this project.

Two categories of sorptive materials, that is, *hydrophilic* and *hydrophobic* ones, have been employed for the purpose of SPE in SI-LOV system.

Thus, the analytical performance of the hydrophilic chelating Sepharose, containing iminodiacetate groups as functional entities on a support of cross-linked agarose, as used in an online SI-LOV system for the determination of ultra-trace levels of Cd(II), Pb(II) and Ni(II) in biological and environmental samples employing electrothermal atomic absorption spectrometry (ETAAS) detection has been investigated. The approach developed exhibits not only high preconcentration efficiency, but due to the low hydrodynamic impedance it allows also easy handling

of the beads.

In comparison with hydrophilic beads, the hydrophobic ones, which inherently adsorb non-charged species, potentially offer high selectivity due to the possible intelligent selection of the chelating reagent used for the formation of the neutral compounds. Hence, SI-LOV approaches using various hydrophobic beads have been developed.

In an earlier study in our group, a PTFE material (Aldrich PTFE beads) was, as compared to other hydrophobic materials, shown to yield excellent performance for adsorption of neutral complexes of transition metals and chelating reagents. However, its inherent physical and morphological characteristics made this material difficult to manipulate in the SI-LOV system. Therefore, a novel PTFE material, granular Algoflon®, which is spherical and possesses higher hydrophobicity, was investigated. The operational characteristics of this sorbent, employed for the determination of Cd(II), as complexed with DDPA and using detection by ETAAS, in a SI system furnished with an external packed column reactor was evaluated and compared with a SI-LOV system using a renewable column. In comparison with the previously used PTFE beads the Algoflon® beads exhibited much higher sensitivity, better retention efficiency and enrichment factor. Moreover, no flow resistance was encountered under the experimental conditions used.

However, the aforementioned approach utilizing hydrophobic sorbents to collect on-line generated neutral compounds is not directly applicable when slow kinetics in the formation and/or adsorption of the non-charged chelates are encountered. Thus, a new concept involving the use of C_{18}-PS/DVB beads, which were preimpregnated with a selective organic metal chelating agent prior to the automatic manipulation of the beads in the microbore conduits of the LOV unit for the determination of trace metals, was conceived. By adapting this approach, the immobilization of the most suitable chelating agent can be effected irrespective of the kinetics involved, optimal reaction conditions can be employed for the immobilization procedure and for implementing the chelating reaction of the measurand with the immobilized reagent, and by using the bead renewal scheme an added degree of freedom is obtained,

allowing the selection of the most favorable elution mode in order to attain the highest sensitivity. A SI-LOV-ETAAS system, using 1,5-diphenylcarbazide (DPC)-coated C_{18}-PS/DVB beads, was successfully applied to the determination of Cr(VI) in natural waters containing high levels of dissolved salts.

Slow kinetics can also be encountered during the process of the generation of the chelate before its adsorption on the beads, as illustrated in the on-line formation of the non-ionic coordination compound between Ni(II) and dimethylglyoxime (DMG) and its collection on a bead material consisting of a reversed-phased copolymeric sorbent with a balanced ratio of hydrophilic and lipophilic monomers, as aimed for the determination of nickel in saline matrixes via a SI-LOV-ETAAS system. Thus, simple on-line mixing of the reactants did not result in any retention of Ni(II), indicating that the formation of the $Ni(DMG)_2$ chelate was slow, and therefore a delay time for its generation and subsequent adsorption had to be incorporated. Thus, to assure sufficient reaction time a reaction coil attached to one of the external ports of the LOV was employed to stack the mixture of sample and reagent for a reproducible period of time prior to the exposure to the beads. The sorbent material exhibited not only superior reversed-phase retention capacity, but also entailed a trouble-free handling in the SI-LOV micro-conduits. The proposed methodology showed high tolerance to the commonly encountered alkaline earth matrix elements in environmental water.

Taking advantage of being readily able to control the kinetic conditions, a SI-LOV-ETAAS system using SPE with hydrophilic chelating Sepharose beads was further proposed for the automatic preconcentration and speciation analysis of Cr(III) and Cr(VI). Exploiting on-line reduction of Cr(VI) to Cr(III), the aspirated sample was initially divided into two portions, which were treated simultaneously. Thus, while the Cr(III) ions in the first portion were subjected to a separation/ preconcentration procedure on the beads, elution and subsequent quantification by ETAAS, the Cr(VI) ions in the second portion were mixed with a reducing reagent and parked under stopped-flow conditions for a reproducible period of time in an open tubular reactor attached to one of the peripheral ports of the LOV unit. Following quantification of the native Cr(III), the Cr(III) formed from Cr(VI) plus the

original Cr(III) was subjected to the same separation/preoncentration/elution procedure. The proposed method was successfully applied to the speciation and determination of trace levels of Cr(III) and Cr(VI) in environmental samples.

The versatility of SI was also demonstrated by its application for in-line microcolumn soil extraction under simulated environmental scenarios and accurate monitoring of ultra-trace levels of readily bioavailable Cr(VI) in soil environments. The SI-LOV system, as attached with a specially designed soil column at one of the peripheral ports of the LOV unit, integrates dynamic leaching of the Cr(VI), on-line pH adjustment, separation/preconcentration of the Cr(VI) by a Q-Sepharose strong anion-exchanger, elution and ultimate detection by ETAAS.

Finally, the LOV was proposed for the separation and preconcentration of metalloids coupled with atomic fluorescence spectrometry (AFS) detection. This was made feasible by interfacing it with a multisyringe flowing stream network for on-line post column derivatization of the eluate aimed at the generation of hydride species. The potential of this new hyphenated technique for environmental assays was ascertained via the determination of ultra-trace level concentrations of total inorganic arsenic in freashwater. Demonstrated for the assay of As, the method involved quantitative oxidation of As(III) to As(V) in the sample before loading into the LOV unit, preconcentration of As(V) on a renewable anion exchanger, pre-reductive elution, mixing of eluate with reducing reagent for hydride generation and subsequent quantification by AFS. Maximum benefit can be taken from the application of the bead renewable strategy, because the application of high concentrations of reductant and extreme pH conditions for the elution prevents the sorbent to be re-used due to the gradual deactivation of the functional moieties. The proposed procedure featured high tolerance to metal species and interfering hydride forming elements.

Sammenfatning (Abstract in Danish)

Ved kemisk analyse af naturlige prøver, som indeholder lave eller meget lave af koncentrationer af measurand, er det ofte nødvendigt at gennemføre en forbehandling – specielt hvis den anvendte detektionsenhed ikke er meget følsom – såfremt prøvematricen er kompleks og kan interferere på selve analysen. Blandt de mange tilgængelige forbehandlingsteknikker er anvendelsen det såkaldte sequential injection lab-on-valve system, den tredje generation af flow injection, formentlig det mest lovende. Denne ph.d. afhandling sætter fokus på at udnyttelsen af alsidigheden i sequential injection lab-on-valve (SI-LOV) systemet med henblik på udviklingen af robuste, automatiske SI-LOV forbehandlingsprocedurer under anvendelse af fast-fase ekstraktion (SPE) til separation og opkoncentrering af sporstoffer i miljøprøver, koblet til diverse atomspektrometriske detektionsteknikker. Idet der kan drages fordel af SI-LOV systemets præcise og reproducerbare timing samt alsidighed, og at det anvendte fast-fase bead-materiale kan fornyes, når det er påkrævet, er der specielt lagt vægt på den intelligente udnyttelse af samspillet mellem termodynamik og kinetik for de involverede reaktioner, d.v.s. på muligheden for at udføre kinetiske diskriminationsprocedurer, som på sin side har resulteret i udvikling af en række originale koncepter, hvilket er illustreret gennem de procedurer, som detaljeret er beskrevet i de videnskabelige publikationer, som projektet har affødt.

To kategorier af sorptive materialer, d.v.s. *hydrofile* og *hydrofobe*, er blevet anvendt med henblik på anvendelse til SPE i SI-LOV systemer.

Således er den analytiske kapacitet af det hydrofile chelaterende materiale Sepharose, som indeholder iminodiacetat-grupper som funktionelle enheder på en basis af krydsbundet agarose, og anvendt i et on-line SI-LOV system til bestemmelse af ultra-lave niveauer af Cd(II), Pb(II) og Ni(II) i biologiske matricer og miljøprøver under anvendelse af elektrotermisk atomabsorptionsspektrometri (ETAAS), blevet nøje undersøgt. Den herved udviklede metode udviser ikke blot høj opkoncentreringseffektivitet, men på grund af den lave hydrodynamiske modstand

tillader den tillige let og bekvem manipulation af de benyttede beads.

I sammenligning med hydrofile beads, så kan de hydrofobe – som selvsagt adsorber ikke-ladede specier – potentielt indebære opnåelsen af høj selektivitet via intelligent valg af det chelaterende reagens til dannelse af de neutrale forbindelser. Af samme årsag er der udviklet SI-LOV metoder under anvendelse af forskellige slags hydrofobe beads.

I en tidligere undersøgelse i vor gruppe blev der afprøvet et PTFE-materiale (Teflon), Aldrich PTFE beads, hvilket i sammenligning med andre hydrofobe materialer viste fortræffelige egenskaber til adsorption af neutrale komplexer dannet af overgangsmetalioner og chelaterende reagenser. Dets iboende fysiske og morfologiske karakteristika gjorde imidlertid, at dette materiale var vanskeligt at manipulere i SI-LOV systemet. Derfor blev et nyt PTFE-materiale, benævnt granular Algoflon®, som er kugleformet og besidder større hydrofobicitet, undersøgt. De operationelle karakteristika for dette sorbent, som anvendt til bestemmelse af Cu(II) ved kompleksdannelse med DDPA og under anvendelse af ETAAS detektion, blev dels undersøgt i et SI system med en ekstern pakket kolonnereaktor og dels, som sammenligning, i et SI-LOV system med regenererbar kolonne. Set i forhold til det tidligere benyttede PTFE-materiale udviste Algoflex® bead-materialet meget højere sensitivitet, bedre retentionseffektivitet og højere berigelsesfaktor. Desuden blev der ikke observeret nogen flow-modstand under de eksperimentelle betingelser, der blev anvendt.

Det ovennævnte tiltag med at benytte hydrofobiske sorbenter til on-line at opsamle de genererede neutrale forbindelser er imidlertid ikke direkte anvendeligt, såfremt dannelsen af og/eller adsorptionen af de ikke-ladede chelater udviser meget langsom kinetik. Af samme årsag blev der udviklet et nyt koncept til bestemmelse af sporstofkoncentrationer af metaller, hvilket involverede anvendelsen af C_{18}-PS/DVB beads, som blev præimpregneret med et selektivt organisk metal-chelaterende reagens inden de pågældende beads blev eksponeret til manipulation i LOV-enhedens mikrokanaler. Ved at benytte denne fremgangsmåde kan immobiliseringen af det mest hensigtsmæssige chelaterende reagens realiseres uanset den involverede kinetik,

optimale reaktionskonditioner kan anvendes såvel ved selve immobiliseringen som ved gennemførelse af den chelaterende reaktion mellem measurand og immobiliseringsreagens, og ved at kunne drage fordel af regenerering af bead-materialet erholdes en ekstra frihedsgrad til valg af den mest favorable elueringsmåde med henblik på at opnå den højeste sensitivitet. Et SI-LOV-ETAAS system under anvendelse af C_{18}-PS/DVB beads pålagt 1,5-diphenylcarbazid blev således succesfuldt benyttet til bestemmelse af Cr(VI) i naturlige vandprøver indeholdende høje niveauer af opløste salte.

Langsom kinetik kan også være tilfældet ved selve processen med at generere chelatet inden dets adsorption på de benyttede beads, som illustreret ved on-line dannelsen af den ikke-ioniske koordinationsforbindelse mellem Ni(II) og dimethylglyoxime (DMG) og dets efterfølgende opsamling på et bead-materiale bestående af et omvendt-fase copylomert sorbent indeholdende et balanceret forhold af hydrofile og lipofile monomerer, hvilket blev benyttet til bestemmelse af nikkel i saltholdige matricer i et SI-LOV-ETAAS system. Indledningsvis blev det således observeret, at en simpel on-line opblanding af reaktanterne ikke medførte nogen retention af Ni(II) på sorbenten, indikerende at dannelsen af $Ni(DMG)_2$ er en langsom proces, hvorfor en tidsforsinkelse for dets generering og efterfølgende adsorption måtte inkorporeres i analyseproceduren. For således at sikre tilstrækkelig reaktionstid blev der tilføjet en reaktionscoil til en af de eksterne porte på LOV-enheden, hvori blandingen af prøve og reagens kunne placeres i en reproducerbar tidsperiode inden eksponering til selve bead-materialet. Dette materiale udviste ikke blot overlegen omvendt-fase retentionskapacitet, men medførte også fuldstændig problemfri manipulation i SI-LOV enhedens mikrokanaler. Den foreslåede fremgangsmåde udviste høj tolerance over for almindeligt forekommende jordalkalimetaller i miljøvandprøver.

Eftersom man således kan drage fordel af at kunne kontrollere de kinetiske konditioner, blev der efterfølgende benyttet et SI-LOV-ETAAS system med SPE v.hj.a. hydrofile chelaterende Sepharose beads til automatisk opkoncentrering og speciering af Cr(III) og Cr(VI). Idet det blev udnyttet, at Cr(VI) on-line kan reduceres

til Cr(III), blev den aspirerede prøve indledningsvis delt i to portioner, hvilke blev behandlet simultant. Medens Cr(III) i den første portion således blev eksponeret til separations-/opkontreringsprocedure på sorbentet, eluering og sluttelig kvantificering v.hj.a. ETAAS, så blev Cr(VI) i den anden portion blandet med reducerende reagens og i en reproducerbar tidsperiode parkeret under stopped-flow konditioner i en åben tubulær reaktor påsat en af LOV-enhedens periferiske porte. Efter kvantificering af det native Cr(III), blev det Cr(III), som var genereret ved reduktionen af Cr(VI) plus det originale indhold af Cr(III) eksponeret til den tilsvarende separations-/opkoncentrerings-/elueringsprocedure. Den foreslåede metode blev succesfuldt anvendt til speciering og bestemmelse af sporstofkoncentrationer af Cr(III) og Cr(VI) i miljøprøver.

SI's alsidighed blev også demonstreret gennem dets anvendelse til in-line ekstraktion af jordprøver, placeret i en mikrokolonne, under simulerede miljømæssige scenarier og nøjagtig monitering af ultra-lave niveauer af direkte biotilgængeligt Cr(VI) i de pågældende jordprøver. Det anvendte SI-LOV system, hvor den special-designede ekstraktionskolonne også her var tilføjet til en af de periferiske porte, tillod således fuld integration af dynamisk udvaskning af Cr(VI), on-line pH-justering, separation/opkoncentrering af Cr(VI) via en stærk Q-Sepharose anion-bytter, elutering, og i sidste ende detektion ved ETAAS.

Slutteligt er LOV systemet blevet anvendt til separation og opkoncentrering af metalloider koblet til detektion v.hj.a. atomfluoroscensspektrometri (AFS). Dette blev faciliteret ved at interface systemet med en flow-manifold, omfattende en stempelpumpe med flere individuelle beholdere, med henblik på at foretage post-column derivatisering af det erholdte eluat og påfølgende hydridgenerering. Potentialet for denne nye sammenkoblede teknik til analyse af miljøprøver blev verificeret ved bestemmelse af ultra-lave koncentrationer af totalt uorganisk arsen i ferskvand. Metodemæssigt bestod proceduren således af præ-oxidation af As(III) til As(V), inden prøven blev introduceret i LOV-systemet, opkoncentrering af As(V) på en regenererbar anionbytter, præ-reduktiv eluering, blanding af eluatet med det til generering af hydridet nødvendige reduktionsmiddel samt efterfølgende

kvantificering ved AFS. I dette tilfælde kunne der specielt drages fordel af at kunne benytte strategien med regenererbar kolonne, idet anvendelsen af høje koncentrationer af reduktionsmiddel og ekstreme pH konditioner ved elueringsprocessen forhindrede genanvendelse af det anvendte sorbent, idet dets aktive grupper gradvist blev deaktiveret. Den foreslåede procedure udviste høj tolerance over for tilstedeværende metaller samt interfererende hydriddannende grundstoffer.

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Appendix **1:** Uncertainty budget for the project *"On-line separation and preconcentration of chromium (VI) in water by diphenylcarbazide loaded C18-PS/DVB beads in Sequential Injection Lab-on-Valve followed by measurement with electrothermal atomic absorption spectrometry (ETAAS)"*

Appendix **2:** Paper I
Paper II
Paper III
Paper IV
Paper V
Paper VI
Paper VII
Paper VIII

Abbreviations

AAS	Atomic absorption spectrometry
AC	Affinity chromatography
AFS	Atomic fluorescence spectrometry
BI	Bead injection
BIS	Bead injection spectroscopy
C_{18}	Octadecyl chemically modified silicagel
C_{18}-PS/DVB	Octadecyl-poly(styrenedivinylbenzene)
CC	Central communication conduit
CE	Capillary eletrophoresis
CL	Communication line
D	Detector
DDPA	Diethyldithiophosphate
DMG	Dimethylglyoxime
DPC	1,5-diphenylcarbazide
EF	Enrichment factor
ESI-MS	Electrospray ionization mass spectrometry
ETAAS	Electrothermal atomic absorption spectrometry
FAAS	Flame atomic absorption spectrometry
FC	Flow cell
FI	Flow injection
FIA	Flow injection analysis
HC	Holding coil
HG	Hydride generation
HPLC	High performance liquid chromatography
i.d.	Inner diameter
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
IV	Injection valve
KR	Knotted reactor
LOC	Lab-on-a-chip
LOD	Limit of detection
LOV	Lab-on-valve
MSFI	Multisyringe flow injection
o.d.	Outer diameter
PEEK	Polyether ether ketone
PP	Peristaltic pump
PTFE	Polytetrafluoroethylene
RC	Reaction coil
RE	Retention efficiency
RSD	Relative standard deviation

SI	Sequential injection
SIA	Sequential injection analysis
SI-LOV	Sequential injection lab-on-valve
SP	Syringe pump
SPE	Solid phase extraction
SV	Multi-position valve
UV/Vis	UV/Visible spectrometry
μTAS	Micro-total analysis system

1. Introduction

1.1 Sample pre-treatment

Nowadays modern instrumental techniques for elemental analysis, such as electrothermal atomic absorption spectrometry (ETAAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma mass spectrometry (ICP-MS), have been widely employed and can meet most of the requirements for trace levels of element determination in the analytical community. However, two challenges are still often encountered in practice: (i) The extremely low concentration of metal species in environmental samples, which is often below the dynamic linear range of the detection instrument; and (ii) the severe spectroscopic and/or non-spectroscopic interferences caused by concomitant matrix components, particularly by high concentrations of electrolytes, that cannot be completely overcome by exploiting existing background correction devices. Therefore, appropriate sample pretreatment involving the separation of the target ions from the interfering matrix constituents, plus a concomitant preconcentration of the analyzed species to fall within the dynamic operational range of the detectors, is often a must.

In this context, efforts have been directed to the development of reliable sample pretreatment methods for measurand isolation/preconcentration prior to sample presentation to the detector. To this end, schemes based on the use of different modern flow injection techniques, such as sequential injection (SI) and its sequel, the so-called sequential injection lab-on-valve (SI-LOV), have been exploited in combination with various atomic spectrometric detectors including flame atomic absorption spectrometry (FAAS), ETAAS, AFS, inductively coupled plasma atomic emission spectrometry (ICP-AES) and ICP-MS for the purpose of achieving enhanced sensitivity and selectivity [1-3]. The fully computer controlled SI and SI-LOV systems, where all unit operations can be effected in an enclosed and automated

fashion and under strictly controlled conditions, have proved to be unique and highly advantageous front-end vehicles to implement such pretreatment methodologies, not only in terms of efficiency, but also in reliability, rapidity and robustness [4- 8]. Additional benefits include that aspiration and propelling of sample and reagent solutions can be effected extremely accurately via the use of the incorporated syringe pump, and that merely minute amount of sample and reagent are required, which in turn means that only small amounts of wastes are generated.

Solid phase extraction (SPE) methods based on liquid-solid extraction has been widely employed in on-line sample processing, not the least because of their high separation and preconcentration efficiency, but also because they can readily be implemented and controlled [9- 12]. Conventionally, SPE-procedures have been implemented by the use of permanent packed column reactors [13], yet in the long run the performance is impaired due to irreversible changes of the surface properties of the solid phase and/or increase of back pressure. However, all these problems can be eliminated by introduction of a new concept called the renewable surface scheme, that is, bead injection (BI), in which the solid phase material, if called for, can be renewed for each analytical cycle, the BI approach being readily incorporated into the SI-LOV system. Its applications in environmental studies [14], immunoassays [15,16], and biological studies [6,17], have shown it to constitute a promising approach for sample pretreatment in all aspects. However, SI-BI-LOV is still in its infancy, yet the exploitation of this methodology to new and unique applications appears most exciting, albeit also challenging. Thanks to the versatility of SI systems, it is expected that this strategy can be extended to more analytical areas.

1.2 Aim of the Ph.D. research project

The overall aim of the Ph.D. research project has been to fully exploit the potential of the SI-LOV system and to develop robust on-line automatic SI/LOV pretreatment

procedures employing SPE for separation and preconcentration of trace elements in environmental samples with various detections such as ETAAS and AFS. Special emphasis has been placed on its application to chemical speciation and dynamic fractionation in environmental solid substrates.

The practical work has been focused on the development of methodologies for on-line pretreatment procedures based on the following schemes:

(i) SI-BI-LOV on-line separation/preconcentration scheme utilizing hydrophilic surfaces for the determination of metal ions by ETAAS. The analytical performance of the system using hydrophilic chelating Sepharose beads as sorbent material is described and discussed.

(ii) SI-BI-LOV on-line separation/preconcentration schemes utilizing various hydrophobic surfaces for the determination of metal ions by ETAAS. Firstly, the operational characteristics of a novel PTFE bead material, used for separation and preconcentration of metal ions via adsorption of on-line generated non-charged metal complexes, are evaluated in a SI system furnished with an external packed column and in a SI-LOV system using renewable scheme. Next, a universal approach based on the use of C_{18}-PS/DVB beads which are preimpregnated with a selective organic metal chelating agent prior to the automatic manipulation of the beads in the microbore conduits of the LOV unit is proposed for selective trace metal determination. Finally, a sorbent containing a balanced ratio of hydrophilic and lipophilic monomers has been introduced for selective trace element separation and preconcentration in complex environmental samples.

(iii) SI-BI-LOV-ETAAS scheme for on-line speciation analysis of trace levels of metal elements. The system deals with on-line reduction and treating two portions of a sample simultaneously.

(iv) SI-BI-LOV-ETAAS scheme for on-line dynamic extraction, separation and preconcentration, and determination of readily bioavailable elements in solid substrates.

(v) Multisyringe flow injection lab-on-valve system coupled to atomic fluorescence spectrometry for on-line preconcentration and determination of

hydride-forming elements in environmental waters.

1.3 Disposition of the thesis

The thesis consists of three parts. The first part consists of Chapter 2 and Chapter 3. Chapter 2 offers a brief introduction to the three generations of flow injection and Chapter 3 covers a state-of-the-art description of techniques applied in SI-LOV systems for on-line sample separation and preconcentration. In the second part, the potential of exploiting the SI-LOV system for sample pretreatment is described and its applications are demonstrated in five subchapters. Chapter 4 and Chapter 5 focus mainly on the development of solid-state clean-up techniques for trace metal element separation and preconcentration exploiting SI-LOV system utilizing hydrophobic and hydrophilic on-line columns reactors. Chapter 6 extends the SI-LOV concept to the application of chemical speciation. In Chapter 7 is described the hyphenation of on-line soil extraction with SI-LOV to acquire enhanced information about mobility and availability of anthropogenic metal species. Chapter 8 deals with the coupling LOV with hydride generation (HG) and atomic fluorescence spectroscopy (AFS) for automated preconcentration/determination of metalloids. The third part includes an uncertainty budget report presented in Appendix 1, which contains the application of a Ph.D. course, namely, *Measurement Uncertainty in Chemical Analysis,* to one of the aforementioned pretreatment schemes for the quantification of measurement uncertainty.

The references cited are listed at the end. The publications which form integral parts of this thesis are marked with Roman number and presented in Appendix 2, while other references are denoted by Arabic number.

2. Historical background of flow injection analysis (FIA)

Flow injection analysis (FIA), as a flow-oriented method of chemical analysis, has been widely accepted in the analytical chemistry community since its first introduction in 1974 [18]. This technique has grown to an important discipline of Analytical Chemistry, which covers a series of monographs and more than 16,000 scientific publications in various journals primarily written in English but also in a multitude of languages as well [19]. The original version of FIA has undergone certain changes and modifications, which can be classified as the three generations [20]: FIA as the first one, sequential injection analysis (SIA) [21] as the second one and lab-on-valve (LOV) [6] as the third one. There are also novel approaches like multicommutation and multisyringe flow injection worth to mention. A brief historical account, principles of operation and individual advantages and limitations of these are presented in the following.

2.1 The first generation of FIA

The first generation of FIA is characterized by the use of a multi-channel peristaltic pump, a two-position injection valve, a coiled reactor and a detector. A typical FIA manifold is depicted in Fig. 2-1. In operation, a volume of sample is loaded into the sample loop of the injection valve while a stream of carrier and a stream of reagent are mixed at the confluence point and flowing continuously through the detector. After the sample loop is filled with the sample, the valve is rotated so that the sample is injected into the constantly flowing carrier stream and transported to the confluence point where it merges with the reagent. During the course of its transport, as a consequence of axial and radial dispersion, the sample reacts with the reagent and a

concentration gradient of detectable species is formed. The detectable species give a transient peak when it passes through the flow cell of the detector.

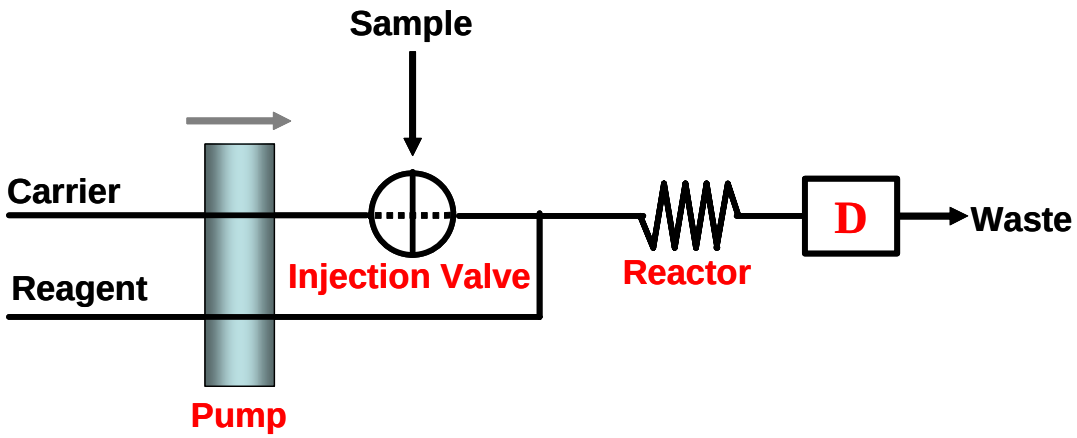


Fig. 2-1. A typical FIA manifold. D, detector.

FIA offers several advantages, such as high sample throughput, straightforward configuration, easy operation, low costs in instrumentation, and compatibility with almost any detection principle [22- 25]. It work continuously and any number of additional lines with reagents can be added. The closed-system chemistry not only reduces the risk of contamination of the sample but also prevents operators contact with hazardous chemicals. In addition, the highly reproducible mixing of streams, controllable dispersion and timing facilitate novel kinetic applications never feasible or thought of before [26].

However, the incompatibility of the elastic tubes of peristaltic pumps with concentrated acids/bases and organic solvents usually necessitate periodically recalibration of the system or the incorporation of more expensive reagent-resisting tubing. Another disadvantage of FIA is that the continuous operation might lead to excessive use of reagents, hence lead to much production of waste.

2.2 The second generation of FIA: Sequential injection analysis (SIA)

In 1990, a variant of FIA, the so-called sequential injection analysis (SIA) was introduced by Ruzicka and Marshall [21]. In comparison with the first generation of FIA, which is based on continuous, uni-directional pumping of carrier and reagent streams, SIA employs programmable, bi-directional discontinuous flow as precisely coordinated and controlled by a computer [5,27]. A typical SIA manifold is illustrated in Fig. 2-2.

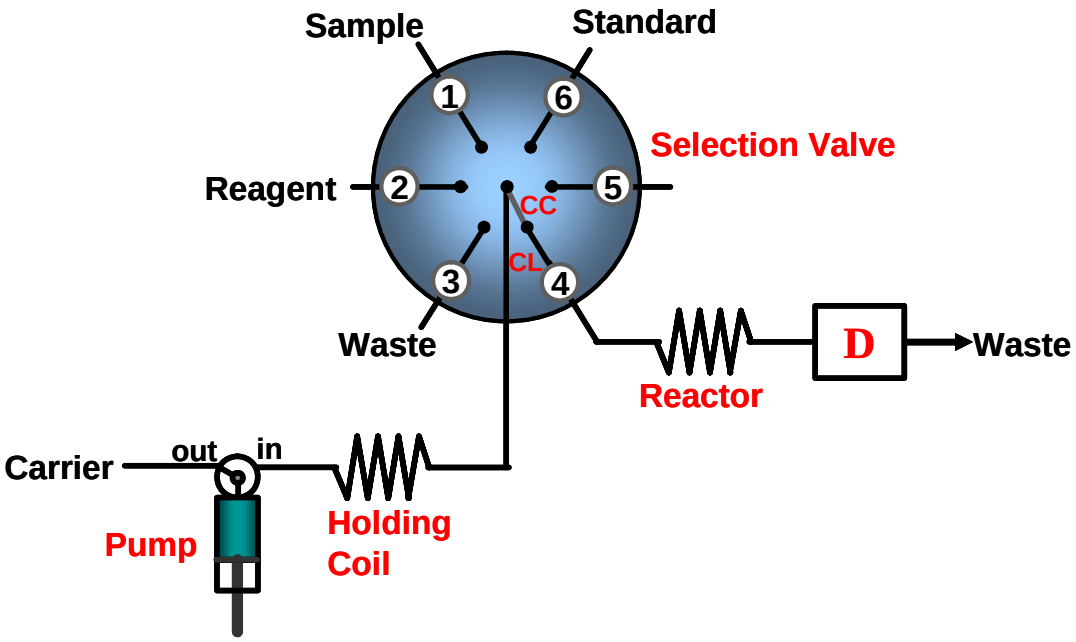


Fig. 2-2. A typical SIA manifold. CC, central communication conduit; CL, central communication line; D: detector.

The heart of an SIA manifold is 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 and reagents, detector and other peripheral units, 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 regent 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.

Instead of the commonly used multi-channel peristaltic pump in FIA, a more accurate, robust syringe pump is employed in SIA as the liquid driver, which allows manipulation of sample and reagent volumes at the low μL level with high precision, and reproducibly permits flow reversals and exploitation of stopped-flow techniques in various approaches for manipulation of the sample.

The notable advantages 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 one. 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 FI/SI techniques in which additional reagents are added downstream by auxiliary syringe pumps [28,29]. 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 before the actual measurement [30].

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. 2-3.

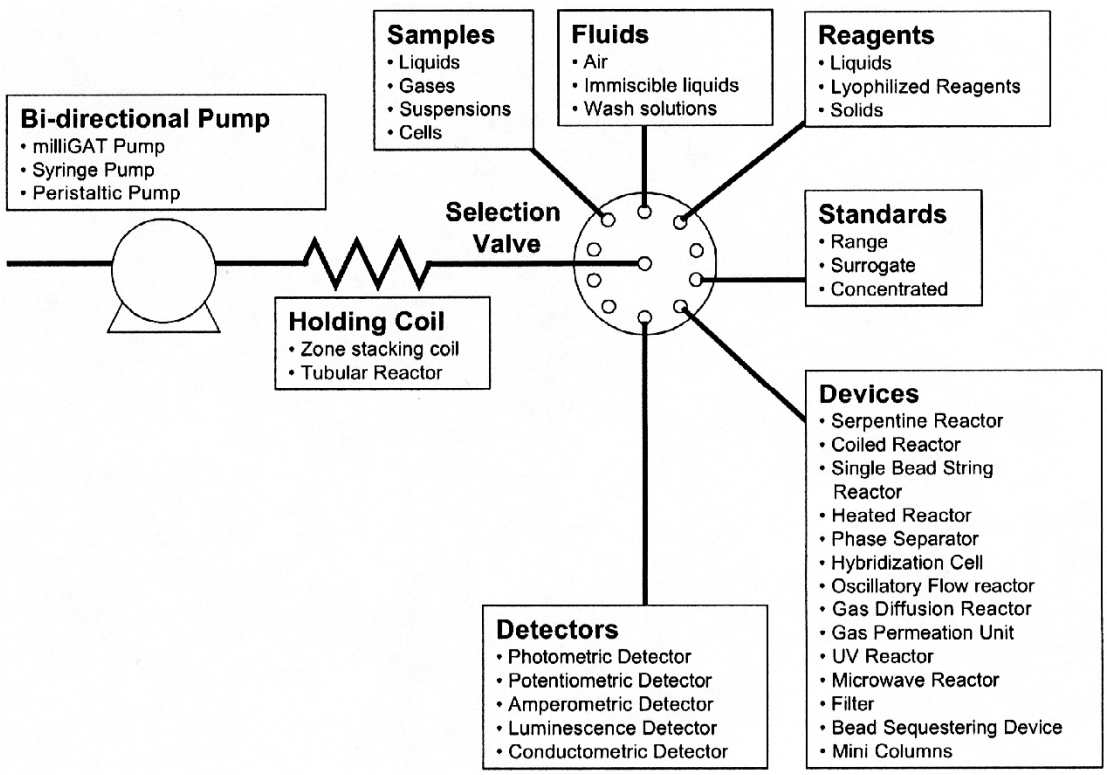


Fig. 2-3. Potential of SIA for automated sample pretreatment (reprinted from ref. [30] by courtesy of Elsevier).

2.3 The third generation of FIA: Lab-on-valve (LOV)

2.3.1 LOV system

The third generation of FIA, lab-on-valve (LOV) was introduced in 2000 [6] as a supplement for SIA, encompassing many features of SIA. Besides the aforementioned components of a SIA system (a multi-position selection valve, a holding coil and a syringe pump), an integrated microconduit, which is normally fabricated by hard PVC furnished with a common central channel corresponding to the central port and

channels corresponding to the ports of selection valve, is mounted atop of the multi-position selection valve. A basic LOV manifold is depicted in Fig. 2-4. 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 micro- and submicro-liter levels to normal ranges that are employed in conventional FI/SI operations.

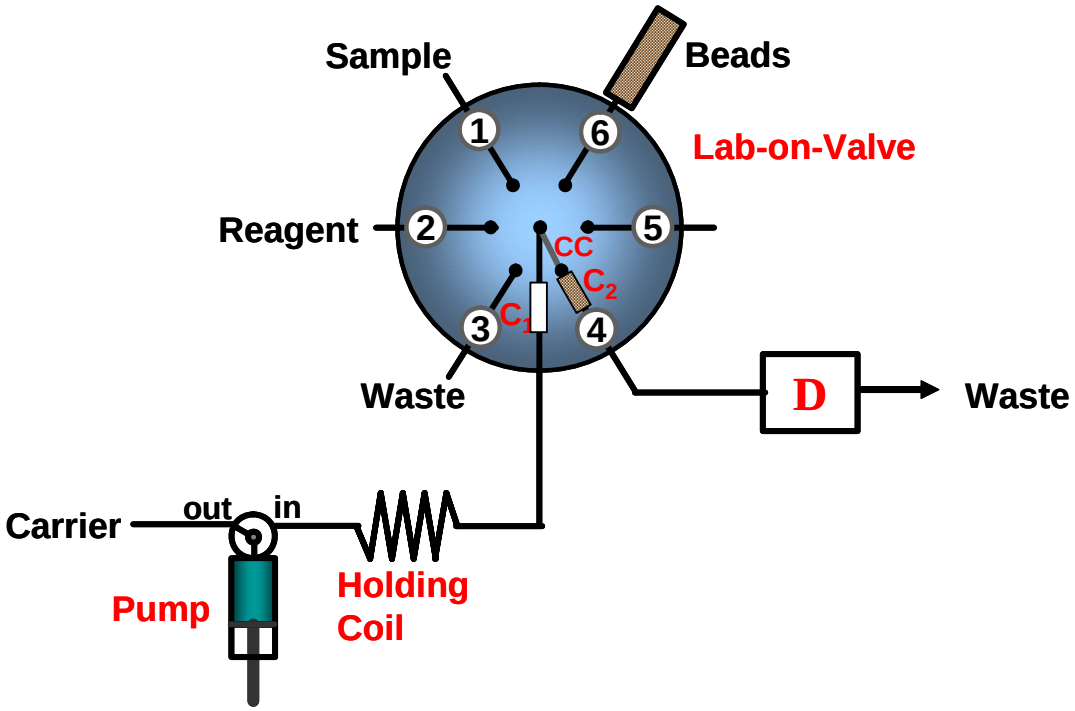


Fig. 2-4. A typical LOV manifold. CC, central communication conduit; C_1, micro column position 1; C_2, micro column position 2; D: detector.

As result of the versatilities of SI, LOV may contain all necessary laboratory facilities such as connecting ports, working channels, solid column reactors packed with small beads furnished with active groups and even detection facilities. In-valve detection by UV/Visible spectrometer or fluorometer are 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 [31], as illustrated in Fig 2-5. 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 measurand intelligently into the detector. In addition, the operation of the selection valve and the syringe pumps are programmable and fully computer controlled. Therefore, it is readily possible to devise different assay protocols in the same manifold.

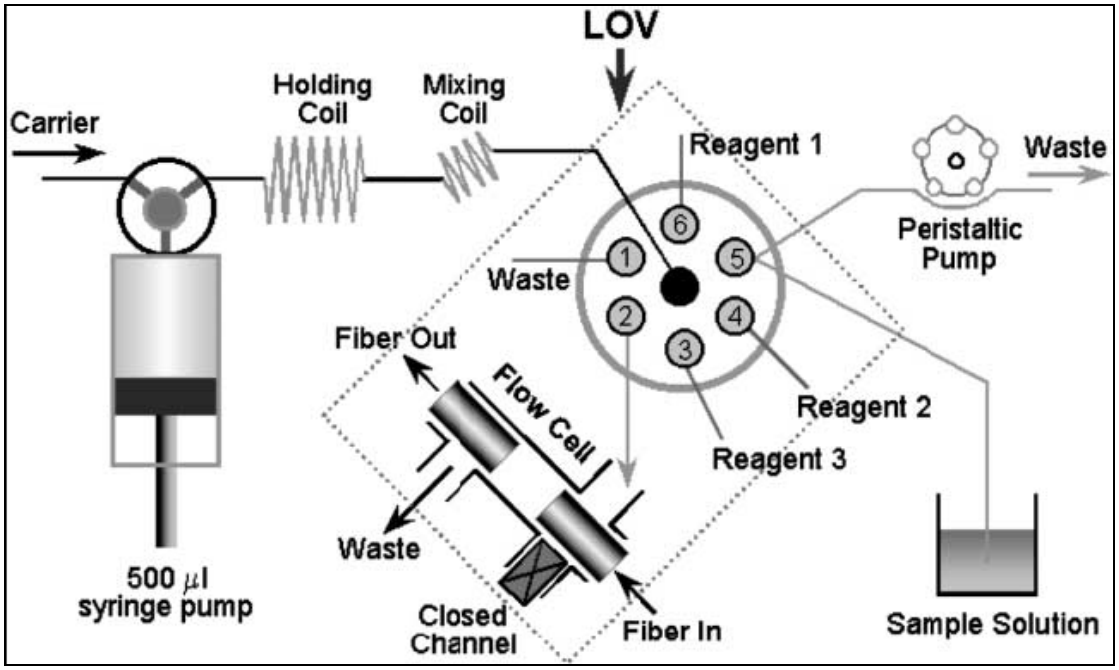


Fig. 2-5. Schematic diagram of a μSI-LOV microsystem incorporating a multipurpose flow cell configured for real time measurement of absorbance (reprinted from ref. [32] by courtesy of Marcel Dekker, Inc.).

2.3.2 LOV vs. lab-on-a-chip (LOC)

Lab-on-a-chip (LOC), also termed the micro-total analysis system (μTAS)[33], is a microfluidic analytical system which automates all necessary processing steps, including sampling, transport, filtration, dilution, chemical reactions, separation and detection, and perform them in a chip. The notable advantage of LOC is that the consumption of expensive and/or rare reagents/samples can be readily reduced to the

nanoliter or sub-nanoliter scale.

In comparison with the micro scale in LOC microsystem and macrosopic world in conventional processed samples, LOV is a mesofluidic analytical system [34] and very suitable for sample processing and manipulation of fluidic/microcarrier beads on a micro-scale, i.e. at microliter to sub-miroliter levels.

The most significant advantage of LOV over LOC lies in its better control of the kinetic conditions of reaction occurring in the system. Techniques such as flow reversal, stopped-flow and incubation can be readily effected in the computer controlled SI-LOV system. This is most preferable for the slow kinetic reaction. Besides, precise and reproducible timing in the system facilitates the exploitation of kinetic discrimination schemes. Therefore, LOV extends the scope of its application to those reactions which are possibly thermodynamically favourable but kinetically unfavorable. In fact, it is exactly these unique facilities which are exploited for the procedures described in this thesis.

2.3.3 Application of sequential injection lab-on-valve (SI-LOV) system

The SI-LOV system, mainly using UV/Visible spectrophotometer or fluorometer furnished with optical fibers as detector, has been applied to bioanalytical assays, environmental monitoring, and elemental analysis. It has also been used as a front-end to instrumentation such as capillary electrophoresis (CE) and hydride generation (HG). Table 2-1 summarizes various applications of the SI-LOV to date.

A SI-LOV approach for simultaneous spectrophotometric determination of copper and iron has been devised [35].

To increase the sensitivities for reactions with slow kinetics, stopped flow technique has been adopted in the SI-LOV system and has been employed for the determination of phosphate [6,36], enzymatic assays [6], and fermentation monitoring of ammonia, glycerol, glucose and free iron [37].

The assay cycle of SIA has been greatly accelerated by simultaneously processing two sample injections within the same manifold [38]. Thus, the average assay time for

a single run has been shortened from 200 s to around 30 s. The approach has been tested on enzymatic assays of glucose and ethanol, but it is, in principle, applicable to all SI reagent-based assays.

Table 2-1. The application of SI-LOV system for bioanalytical assay, environmental monitoring, elemental assay and as a front-end to CE and HG.

System	Applications	Detection	Reference
SI-LOV	Copper, iron	Absorbance	[35]
SI-LOV	Phosphate	Absorbance	[6, 36]
SI-LOV	Enzymatic assay	Fluorescence	[6]
SI-LOV	Fermentation monitoring of ammonia, glycerol, glucose and free iron	Absorbance	[37]
Accelerated SI-LOV	Enzymatic assays of glucose and ethanol	Absorbance	[38]
μSI-LOV meso-fluidic system	DNA assay	Absorbance	[39]
μSI-LOV meso-fluidic system	DNA assay	Fluorescence	[40]
SI-LOV	Enzyme kinetics and inhibition study: acetylcholinesterase and angiotensin-converting enzyme	Absorbance	[41]
SI-LOV with a cadmium column	Nitrate and nitrite, on-line reduction of nitrate to nitrite	Absorbance	[36]
SI lab-at-valve	Extractive determination of an anionic surfactant	Absorbance	[42]
SI-LOV-CE	Front-end to CE for anion separation	Absorbance	[43]
SI-LOV-CE	Front-end for insulin derivatization/separation	Fluorescence	[44]
SI-LOV-HG	Mercury, coupling SI to HG	Absorbance	[45]

By the employment of a LOV meso-fluidic analytical system, DNA assays were achieved [39,40].

A micro-reactor with continual spectrophotometric detection has been operated in SI-LOV mode and applied to enzyme kinetics and inhibition studies, using acetylcholinesterase (AChE) and angiotensin-coverting enzyme (ACE) as model systems [41].

For the on-line measurement of nitrate and nitrite, a cadmium reduction column was incorporated in the LOV system to reduce nitrate to nitrite before merging the stream of nitrite with chromogenic reagents [36]. Stopped flow technique was

employed to increase the reduction time for improved efficiency.

A lab-at-valve micro-extraction system for the spectrophotometric determination of an anionic surfactant was proposed [42]. A designed component, a separating chamber, was attached at one port of a conventional multiposition selection valve. A spectrophotometer was directly plugged at the separating chamber via an optic fiber so that the detection process could be performed at the valve instead of transport of the product to the flow cell of the spectrophotometer.

The multipurpose flow cell in the SI-LOV system was reconfigured as a front-end to the CE system for anion separation [43] and insulin derivatization/-separation [44]. The SI-LOV system not only provided an efficient means of delivering sample to the CE system with various sample-injection modes, including electrokinetic, hydrodynamic and head column field amplification (HCFA) sample stacking, but also served as a versatile means of sample pretreatment to facilitate the ensuing CE separation.

SI technique was also introduced to HG for mercury detection by cold vapor atomic absorption spectroscopy with advantages of using programmable flow and miniaturization of assays [45].

3. Solid phase extraction in flow systems

3.1 Introduction

A multitude of separation and preconcentration techniques based on batch or flow injection modes have been developed, including solvent extraction [46,47], solvent extraction/back-extraction [5,48], solid phase extraction [49,50-52], precipitation/-coprecipitation [48,50,52,53], or hydride and vapor generation [54-56].

Column-based solid phase extraction (SPE) [57], which employs an appropriate solid material, is among the most efficient and widely employed on-line separation/preconcentration techniques and its incorporation with detection by atomic spectrometry has received extensive attention. This sample processing method has been growing rapidly as a consequence of its straightforward operation and high separation and preconcentration capabilities. The great advantage of SPE is that both organic compounds and inorganic species can be extracted. Depending on the nature of the measurand and on the retention mechanism, various extraction materials have been employed. Moreover, methods of extraction in knotted reactors, where the tubing of the reactor serves as the extraction material, can also be considered as belonging to the family of SPE methods [58-61].

Sorptive materials in solid phase extraction, used for the determination of metal ions down to the sub-ng L^{-1} level, comprise chelating ion exchangers such as Chelex-100, immobilized 8-hydroxyquinoline and dithizone modified Sephadex G-25 [62]; anion and cation exchangers such as Sephadex C-25 [63,64]; activated carbon [65]; C_{18}-silicagel [66,67]; octadecyl-chemically modified poly(styrenedivinyl-benzene) copolymers (C_{18}-PS/DVB) [68]; poly(tetrafluoroethylene) (PTFE) [69,70]. Basically, sorptive materials fall into two categories: hydrophilic [62-64] and hydrophobic [58,60,61,66-70].

The temporary retention of low levels of individual metal ions on the surface of

the sorbent can be achieved by electrostatic interactions onto ion-exchanger packed-bed columns or chelating reactors which generally contain iminodiacetate moieties. They can also be derivatized to non-polar chelates and subsequently retained on reversed-phase materials by partitioning, hydrophobic or π-π stacking interactions.

3.2 The state-of-the-art of sequential injection bead injection lab-on-valve (SI-BI-LOV) scheme

3.2.1 Introduction

SPE material in conventional FI is generally permanently packed in a column which is treated as an integral component of the manifold prior to detection of the measurand. 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 FI 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 [71]. 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) [72], that is, the column is simply renewed or replaced for each analytical run. According to this scheme, the solid phase material, in the forms of beads, is injected and retained in a special cell. This concept is well suited in LOV incorporating a miniaturized renewable column, i.e., SI-BI-LOV.

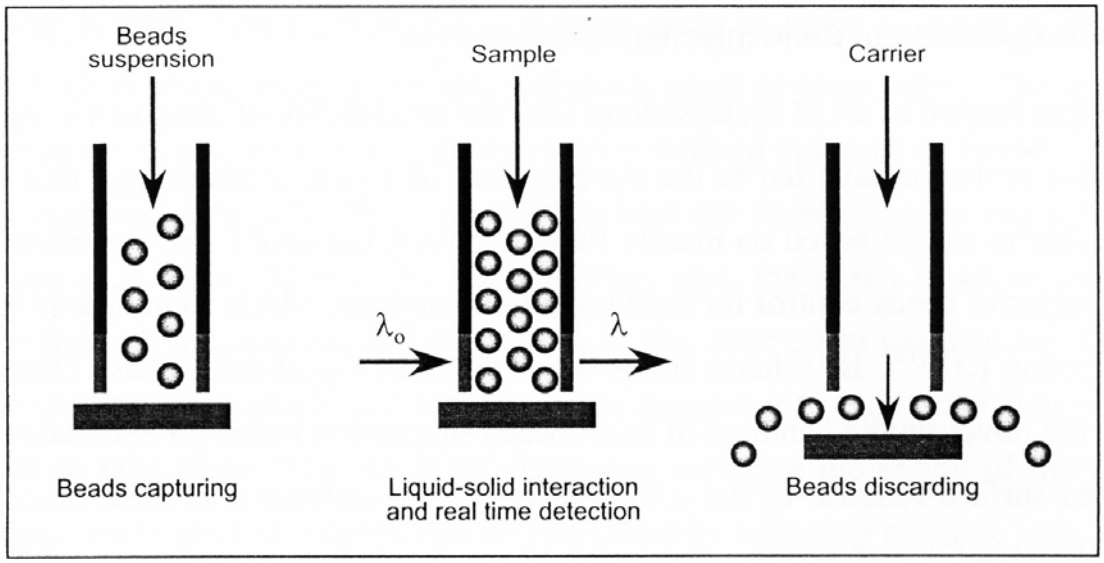


Fig. 3-1. A schematic diagram of Jet-Ring-Cell for bead injection (reprinted from ref. [73] by courtesy of Technical University of Denmark).

One of the most widely used beads surface renewable approaches is the jet-ring-cell [72, 74], which is schematically illustrated in Fig. 3-1. A typical operational procedure includes: beads capturing in the cell, perfusing with sample for solid liquid interaction, treatment of the measurand with auxiliary reagents allowing for detection, and beads discarding at the end of each analytical run. As shown in Fig. 3-2, the LOV integrates the sample processing channels with a multiposition flow cell, with fiber optic UV/Vis spectrophotometer-fluorometer as detectors. The multiposition flow cell can be readily configured exactly as jet-ring-cell, as illustrated in Fig. 3-3, where the change in bead optical properties can be monitored by absorbance (Fig. 3-3D), fluorescence (Fig. 3-3E) or reflectance (Fig. 3-3F)

spectrometry. The beads can be effectively discarded by a short burst of flow reversal into the holding coil and then to waste. This methodology has been demonstrated for bioligand interactions assays of immunoglobulin (lgG) based on its interaction with protein G immobilized on Sepharose beads [6].

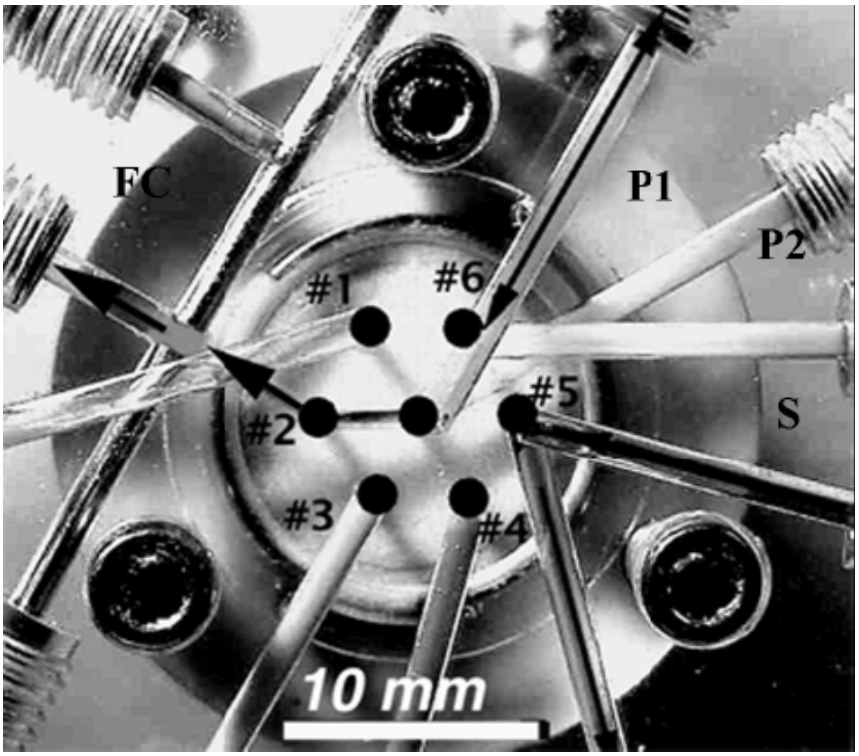


Fig. 3-2. Lab-on-valve shown mounted atop a six position valve. P1, P2 are channels leading to holding coils and syringe pumps. Sample S is shown in a flow through sampling port (#5) that is connected to the sample container and peristaltic pump. The flow cell (FC) is shown in absorbance configuration 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 of measurand into the flow cell (reprinted from ref. [6] by courtesy of The Royal Society of Chemistry).

LOV approach with renewable sorbent in microcolumn for preconcentration/separation have been developed for the determination of metal elements in our group. Whatever the bead material applied, the analysis cycle contains four stages: (i) firstly the microcolumn is packed with a small, well defined volume of bead material. Then (ii) the packed column is loaded with a well defined volume of sample, and the measurand or non-charged compound formed from the measurand and the organic chelating reagent is retained on the column while the matrix goes to

the waste. Thereafter (iii) the retained measurand is eluted by a small volume of an appropriate eluent, which subsequently is transferred to the detector. And finally (iv) the beads are discarded and new beads are aspirated for the next cycle.

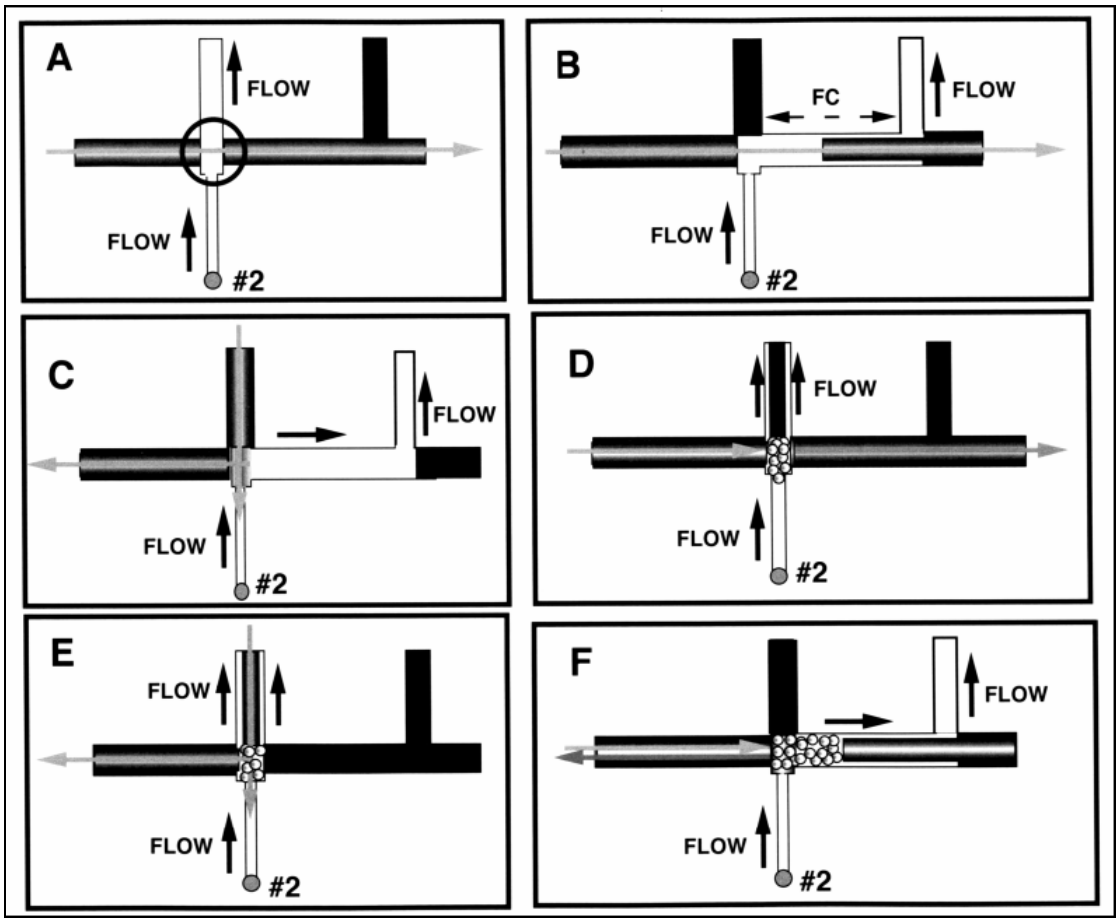


Fig. 3-3. Multipurpose flow cell, which is integrated in LOV, uses optical fibers encased in stainless steel tubing that is proportioned to leave a 30 μm gap between the casing and channel walls. The fibers can be readily reconfigured for absorbance (A, B) and fluorescence (C) measurement. Since the 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 (reprinted from ref. [6] by courtesy of The Royal Society of Chemistry).

In Fig. 3-4 is showed a LOV system employed in our group, in which two of the channels are defined as microcolumn positions (C_1 and C_2). To trap the beads within the channel cavities and to prevent the beads from escaping during the operations, the outlets of these two channels are furnished with small pieces of PEEK tubing, which fit into, and are affixed, by the screws in the outlets. The diameter of this tubing is

slightly smaller than the diameters of the columns. It provides an internal channel which thus allows liquid to flow freely along the walls, but entraps beads. A close-up of the packed renewable microcolumn is shown in Fig. 3-4.

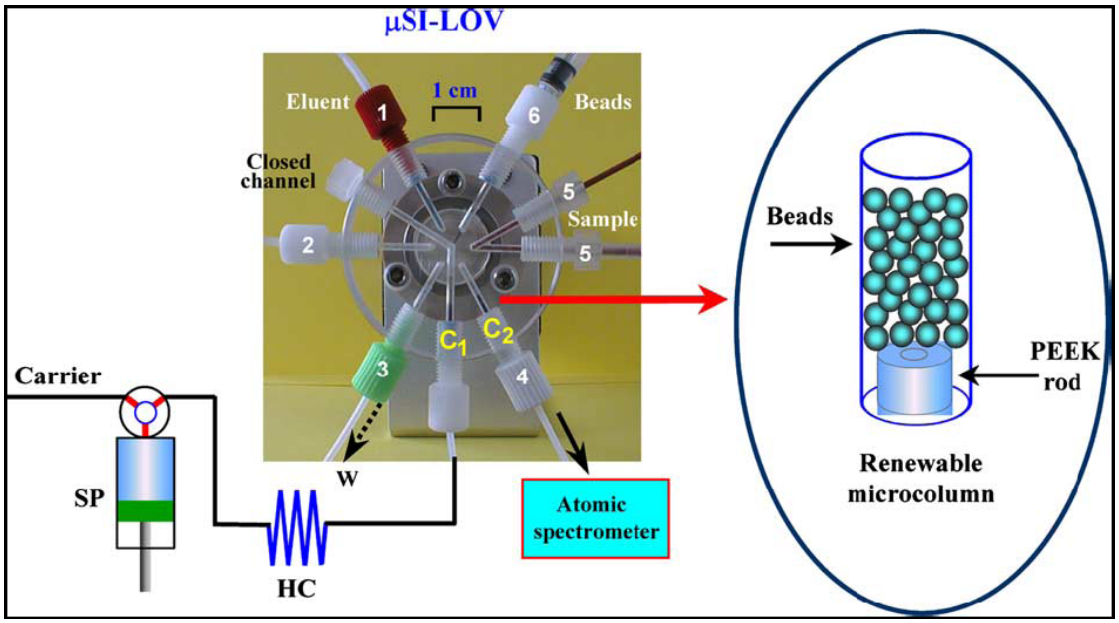


Fig. 3-4. Diagram of a LOV system for bead injection (BI) incorporating two microcolumn positions (C_1 and C_2), along with a close-up of a packed renewable microcolumn (reprinted from ref. [13] by courtesy of Elsevier).

Commercially available beads suspension, mostly stored in 20% ethanol, can be used directly, while the 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). In operation, beads suspension is first aspirated into a 1.0 mL plastic syringe that afterwards is mounted on a port of the LOV. To pack the column precisely and reproducibly for each analytical cycle, a relatively low aspiration rate of beads suspension (normally 5 µL s^{-1}) from the plastic syringe into micro-column position C_1 is preferable. After aspiration, the beads can be transferred back and forward between the two micro-column positions (C_1 and C_2) and finally discard to the waste as the protocol requires.

3.2.2 Measurand-loaded beads treatment

After the beads have been exposed to a certain amount of sample solution, there are two possible approaches [5,64,75,76] for dealing with the measurand-loaded beads, as illustrated in Fig. 3-5. One is to elute the loaded beads with a small, well-defined volume of eluent which is ultimately transported to the detector, whereupon the used beads are discarded to waste. Thereafter, fresh beads are aspirated to generate fresh columns for next assay cycle. An alternative is to directly transport the loaded beads to the detector for quantification. The first approach is the most popular one and matches various detectors such as FAAS, ETAAS, ICPAES and ICPMS, while the latter one can only be applied to ETAAS [5], where advantage can be taken by the fact that the beads consist primarily of organic materials, that is, they can be pyrolyzed thereby allowing ETAAS quantification of the measurands.

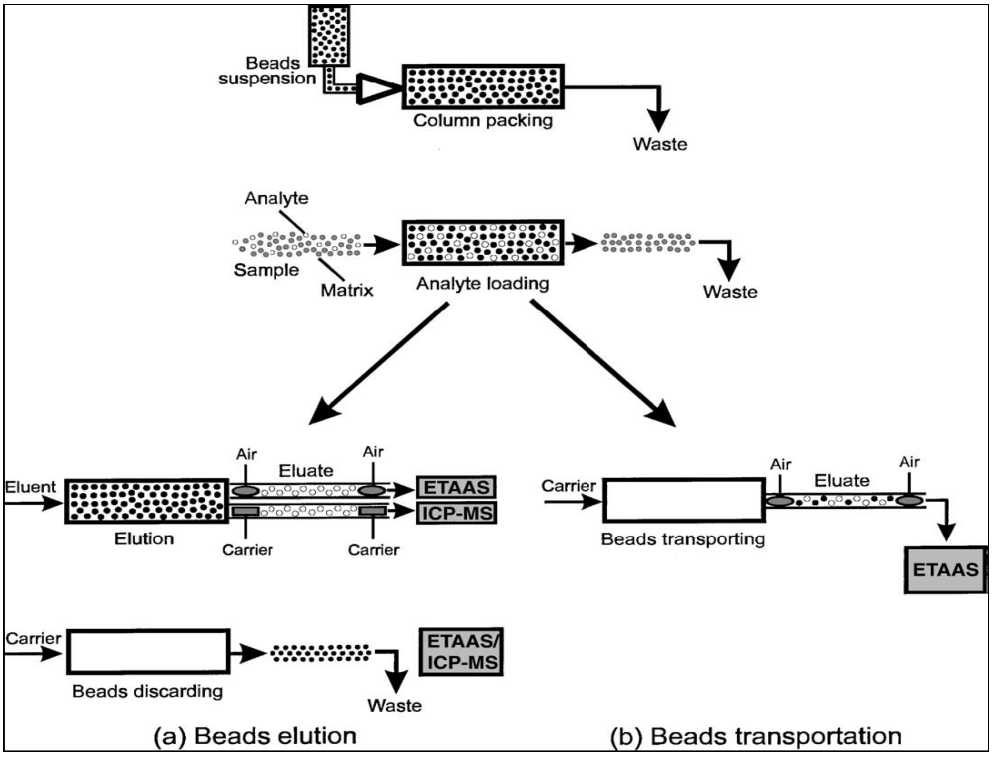


Fig. 3-5. Illustration of two possible schemes for dealing with measurand loaded beads in the renewable microcolumn approach. (a) by eluting the preloaded beads by a defined volume of eluent which then is transferred to ETAAS and/or ICPMS for quantification. (b) by transporting the loaded beads directly into the graphite furnace of the ETAAS instrument (reprint from ref. [5] by courtesy of Elsevier).

The micro-miniaturized renewable column volume in the LOV results in a correspondingly smaller breakthrough capacity for the measurand, and consequently a very limited amount of eluent suffices for the complete elution of the retained measurand. It is, therefore, critical to minimize the dispersion of the eluate zone during its transport to the detector, as this might cause severe loss of sensitivity. The introduction of small air segments at both ends of the eluate zone in the tubing to preserve its integrity might help to minimize the dispersion [77-79]. Thus, in ETAAS, after sample loading, an air zone is transported to the microcolumn position C_2 to empty the tubing leading to the detector. Followed by another air zone, the eluate is thus sandwiched by air segments while it is transported to the detector.

3.2.3 Interface between LOV and detectors

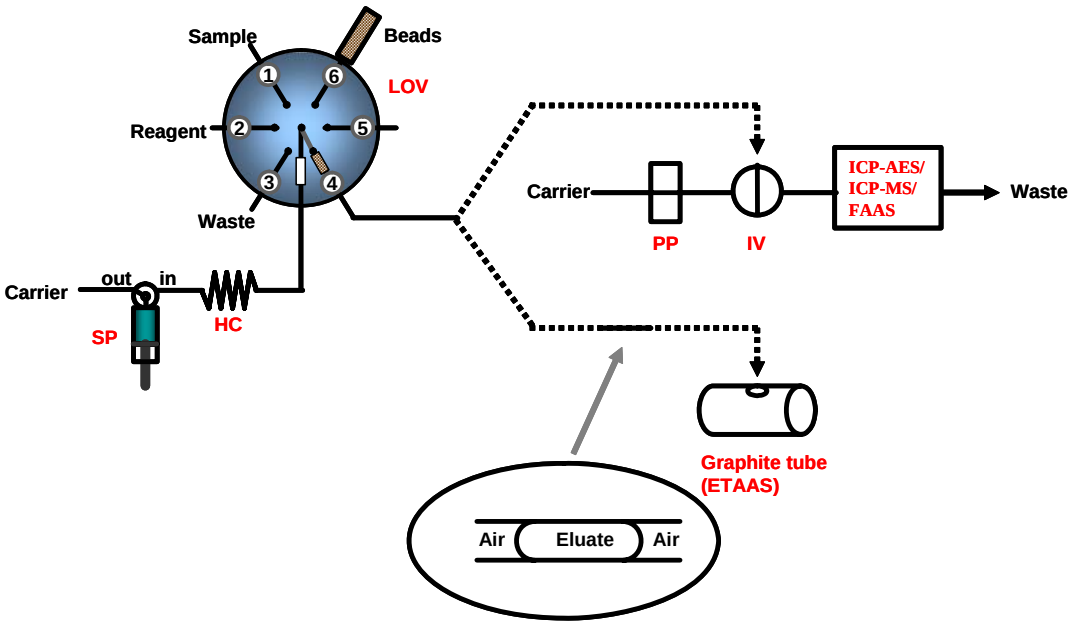


Fig. 3-6. Interface of LOV system with various detection devices, along with a close-up of air-sandwiched eluate in the tubing for ETAAS detection. SP, Syringe pump; HC, Holding Coil; PP, Peristaltic Pump; IV, Injection Valve (adapted from ref. [5] with courtesy of Elsevier).

The interface between LOV and various detectors for trace elements measurement is dependent on the nature of detectors. There are two possible schemes which are

shown in Fig. 3-6. The discontinuous operation of SI makes this technique well suited for hyphenation with ETAAS as a consequence of the discrete, non-continuous working nature of the detector. The stringent volumetric demands (normally less than 50 μL) of the graphite tube of the ETAAS detector are easily met in SI-SPE by automatic programming of an air-segmented elution mode, whereby a discrete, well-defined volume of eluent containing the stripped measurand (i.e., less than 50 μL) is delivered to the atomizer as sandwiched between air plugs.

As for continuous-flow detectors, such as FAAS and ICP-AES or ICP-MS, the interface can be constructed from conventional flow-through sample injectors, such as rotary valves, as reported by several authors [63,80]. In this scheme the eluate can be firstly filled in the sample loop of the injection valve and subsequently transported to the detectors by carrier solution.

3.3 Application of SI-BI-LOV system

The SI-LOV with renewable surface scheme has proven to be a very attractive methodology in many contexts. It has been applied to trace elemental assay, bioanalytical assay and as a front-end to electrospray ionization mass spectrometry (ESI-MS) and high performance liquid chromatography (HPLC) as well. Table 3-1 summarizes various applications of the SI-BI-LOV system to date.

Researches on SI-BI-LOV scheme coupled to ETAAS and ICP-MS detection for automatic on-line sample pretreatments and determination of trace elements have been mainly conducted in our group. Two categories of sorbent beads have been employed to pack renewable microcolumns, that is, hydrophilic SP Sephadex C-25 cation exchanger and iminodiacetate based Muromac A-1 chelating resins, and hydrophobic materials such as poly(tetrafluoroethylene) (PTFE) and poly(styrenedivinylbenzene) copolymer alkylated with octadecyl groups (C_{18}-PS/DVB). The hydrophilic SP Sephadex C-25 cation exchanger (a cross-linked polysaccharide modified with sulphonic groups) was employed for the

Table 3-1. The applications of SI-BI-LOV system

System	Beads material	Application/species assayed	Detection	Reference
SI-BI-LOV	SP Sephadex C-25 cation-exchange resin	Ni, Bi	ETAAS	[64,75,76]
SI-BI-LOV	SP Sephadex C-25 cation-exchange resin	Ni, Bi	ICPMS	[63]
SI-BI-LOV	Muromac A-1 chelating resin	Pb	ETAAS	[81]
SI-BI-LOV	PTFE and C_{18}-PS/DVB	Cd	ETAAS	[68]
SI-BI-LOV	Dithizone impregnated Sephadex G-25	Pb	ETAAS	[82]
SI-BI-LOV	C_{18}-PS/DVB and C_{18}-silicagel	Cd, precipitate	ETAAS	[83]
SI-BI-LOV-HG-AFS	C_{18}-PS/DVB	Cd, co-precipitate	AFS	[84]
SI-BI-LOV	Protein G-coated Sepharose beads	Bioligand interaction assay of IgG	Fluorescence	[6]
SI-BI-LOV	pH indicator immobilized Sephadex® beads and Cytopore® Beads	Monitoring of extracellular acidification rates	Absorbance	[85]
SI-BI-LOV with an microbioreactor	Cytopore® Beads (Uppsala, Sweden)	Monitoring of lactate extrution and glucose consumption of cultured cells	Absorbance	[86,87]
μAC and μBIS	Protein G-coated Sepharose beads	IgG	Absorbance	[88]
BIS	Agarose beads	Monitoring of immobilization of proteins on beads	Absorbance	[89]
SI-BI-LOV-UV/Vis and SI-BI-LOV-ESI-MS	Anion-exchange resin (AG 1-X4 in acetate form)	Monitoring the kinetics of interaction between biotin-containing conjugates and immobilized streptavidin	US/Vis and ESI/MS	[90]
MSFIA-BI-LOV-HPLC	Copolymeric divinylbenzene-*co*-n-vinylpyrrolidone beads (Oasis® HLB)	Determination of five acidic pharmaceutical residues (viz., ketoprofen, naproxen, bezafibrate, diclofenac, and ibuprofen) and one metabolite (viz., salicylic acid)	HPLC	[91]

preconcentraion of Ni, Bi coupled with detection by ETAAS and ICP-MS [63,64,75,76]. At low flow rates (20 μL s^{-1}) beads can be effectively entrapped in the microcolumn positions and can be transferred reproducibly within the LOV system, while at higher flow rate (100 μL s^{-1}) they become squeezed and can flow through the space between the channel and the PEEK stopper tubing, which results in a unique way to discard the used beads. Muromac A-1 beads, a chelating resin containing iminodiacetate groups, were investigated in a similar system for the preconcentration of Pb with the detection by ETAAS [81]. It showed somewhat higher R.S.D.-values due to difficulties in aspiration of reproducible bead volumes. The performance of two kinds of hydrophobic materials, PTFE and C_{18}-PS/DVB, for the preconcentration of cadmium was investigated [68]. The manipulation of C_{18}-PS/DVB beads suspension is as straightforward as that for hydrophilic beads because of their similar morphology and physical properties. On the contrary, PTFE beads have neither a spherical shape nor size-homogeneity. Therefore, it was difficult to aspirate the beads from the beads container and hence not practically useful. However, the PTFE material showed nearly three times as high retention efficiency as that of the C_{18}-PS/DVB.

Dithizone impregnated Sephadex G-25 beads were employed in a renewable column in the SI-BI-LOV system for the determination of trace concentration of lead with detection by ETAAS [82]. The Pb-sorbed beads were directly transferred from the LOV into the graphite tube of the ETAAS for measurement using a mixture of Pd, Mo and tartaric acid as chemical modifier.

Two kinds of octadecyl immobilized beads including C_{18}-PS/DVB and C_{18}-silicagel were proven to be superb media for the collection of cadmium hydroxide precipitate. With a renewable microcolumn in the LOV coupled to ETAAS detection, the system was used for determination of ultra-trace Cd [83].

A SI-BI-LOV system incorporating a renewable microcolumn packed with C_{18}-PS/DVB microbeads was applied for co-precipitation preconcentration of Cd by hyphenation with hydride generation atomic fluorescence spectrometry [84]. Cadmium was co-precipitated with lanthanum hydroxide and collected on a microcolumn in the LOV. The co-precipitate was eluted with hydrochloric acid and

directed to meet tetrahydroborate to facilitate hydride generation. The hydride was separated from the reaction mixture and was swept into the atomizer.

SI-LOV provides itself as an effective approach for the study of cellular activities and function in which live cells can be immobilized on microcarrier beads. Based on bead injection, real-time monitoring of extracellular acidification rates [85], lactate extrusion and glucose consumption of living cells grown on micro carrier beads (Cytopore®) [86,87] are feasible.

Two complementary techniques, micro-Affinity Chromatography (μAC) and micro-Bead Injection Spectroscopy (μBIS), have been applied to the assay of biomolecules using immunoglobulins (human IgG, rabbit IgG, and horse IgG) and protein G-coated beads [88]. Both methods used the same micro-sequential injection instrumentation and bead injection methodology to form renewable micro-columns. μAC monitored the eluted measurand post-column, while μBIS monitored the capture and elution of measurand on-column.

Bead Injection Spectroscopy (BIS) was also used for real time monitoring of the immobilization of proteins on agarose beads by measuring the rate and yield of coupling reactions, as they take place on the surface of agarose beads [89]. Thus, BIS provides a useful tool for quality control of agarose-based chromatographic supports, as well as for the optimization of a wide variety of immobilization chemistries, as used for synthesis of chromatographic supports, immobilization of enzymes, and derivatization of biosensing surfaces.

A method coupling BI-LOV with UV/Vis and electrospray ionization mass spectrometry (ESI-MS) for monitoring the kinetics of interaction between biotin-containing conjugates and immobilized streptavidin has been proposed [90].

The third generation of flow injection analysis as a front-end to high performance liquid chromatography (HPLC) for on-line solid phase extraction sample processing by exploiting the bead injection concept has been presented [91]. The hyphenation of multisyringe flow injection analysis (MSFIA) with BI-LOV prior to HPLC analysis was utilized for on-line postextraction treatment to ensure chemical compatibility between the eluate medium and the initial HPLC gradient conditions. The potential of

the novel MSFI-BI-LOV-HPLC for on-line handling of complex environmental and biological samples prior to reversed phase chromatographic separations was assessed for the expeditious determination of five acidic pharmaceutical residues (viz., ketoprofen, naproxen, bezafibrate, diclofenac, and ibuprofen) and one metabolite (viz., salicylic acid) in surface water, urban wastewater, and urine.

3.4 Characteristic parameters in SI-BI-LOV on-line preconcentration system

A multitude of parameters have been employed for the evaluation of the performance characteristics of SI-BI-LOV on-line preconcentration procedures. The most widely used ones are enrichment factor and retention efficiency.

(i) Enrichment factor (*EF*)

The enrichment factor is theoretically defined as the ratio between the concentration of the measurand in the final solution after preconcentration (eluate), C_e, and that in the original sample, C_s, i.e.:

$$EF = \frac{C_e}{C_s}$$

In practice, the *EF* value is usually obtained from the ratio of the linear range sensitivity of the proposed preconcentraiton method and that obtained by direct detection of standards.

(ii) Retention efficiency (*RE*)

In SPE the retention efficiency is much more straightforward than *EF* in characterizing the performance of a sorbent material. *RE (%)* is defined as the ratio between the retained amount of measurand and the maximum available measurand in the sample. In practice, it can be deduced by the *EF*, assuming quantitative elution of the measurand loaded beads, i.e.:

$$RE(\%) = \frac{EF}{\left(\frac{V_s}{V_e}\right)} \times 100$$

Where V_s and V_e are the volumes of sample and eluent, respectively.

4. SI-BI-LOV-ETAAS schemes utilizing hydrophilic surfaces for the determination of metal ions [II]

4.1 Introduction

The ideal sorbent for SPE in LOV system can be handled like a liquid, that is, it can be easily aspirated into the cavity of the LOV from the beads container to generate the microcolumn and transported from one column position to another one, and finally to the waste.

For that purpose, there are several stringent requirements to be met for the solid phase materials: It should (i) have a perfectly spherical shape, that is, in the form of ball-shaped particles; (ii) possess suitable size (preferable within the range 40-150 μm) resulting in enough total sorption capacity and facilitating the entrapment of beads within the micro column position of the LOV as formed by the stopper as well; (iii) have uniform in size distribution; and (iv) have a density close to that of water.

In the search of ideal sorbents suitable for the SI-LOV mode, commercially available chelating Sepharose, consisting of hydrophilic agarose support with iminodiacetic acid groups is a very promising candidate. The rigid base matrix permits very high flow velocities. The spherical beads have a mean size of 90 μm which is perfectly suited for use and manipulations in on-line systems, not the least because the beads exhibit negligible volume variations due to changes in pH or ionic strength. Besides, the material is chemical stable under both acidic and alkaline conditions. In addition, the hydrophilic properties make it perfectly suitable in aqueous media.

Therefore, chelating Sepharose is proposed as SPE sorbent in the SI-LOV mode for the determination of ultra trace amount of Cd, Pb and Ni in biological and environmental samples. Cd and Pb are highly toxic elements and their concentration in environmental samples, body fluids and tissues are of main concern in the studies

of environmental pollution and occupational exposure [92,93]. Ni is an essential element for human heath, but some of its compounds are carcinogenic [94].

4.2 Method development: Configuration, parameters and operational procedure description

The diagram of the system is schematically shown in Fig. 4-1, in which chelating Sepharose beads suspension is filled in the reservoir (syringe) mounted at port 6 of the LOV [64].

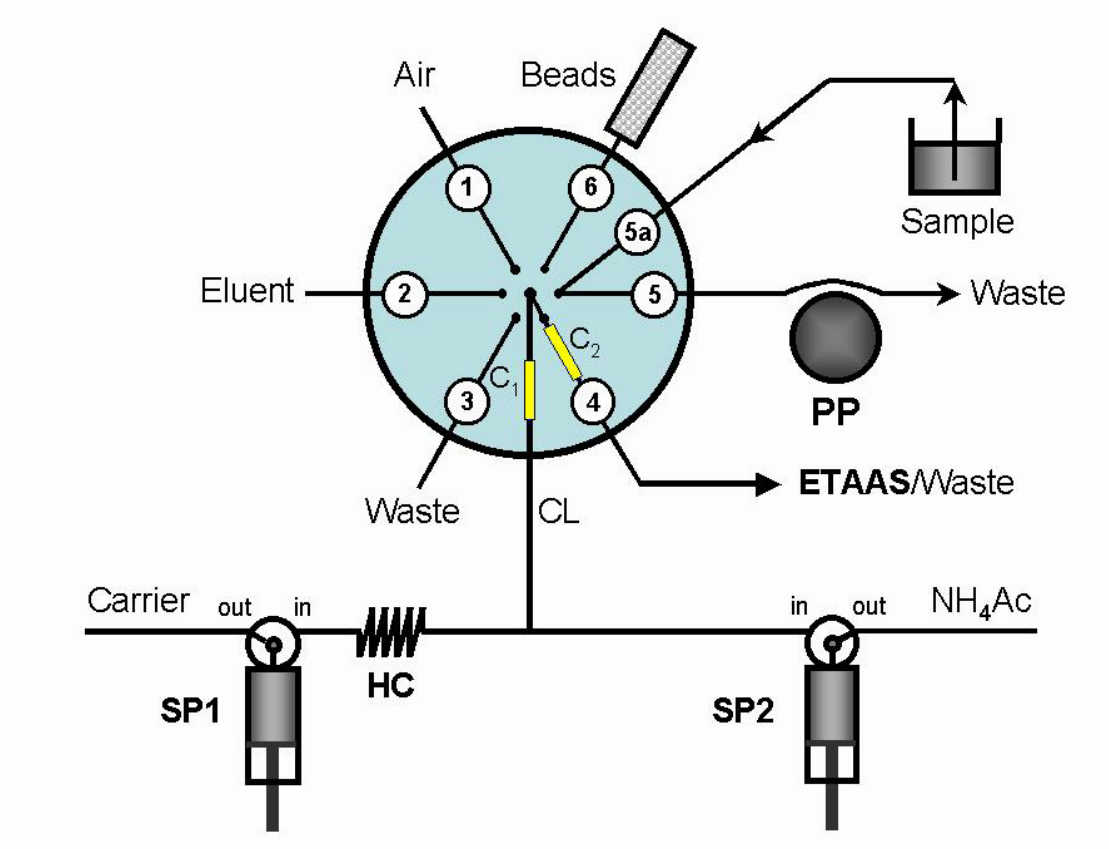


Fig. 4-1. SI-BI-LOV system using chelating Sepharose for determination of ultra-trace levels of Cd, Pb and Ni by ETAAS. SP1 and SP2, syringe pumps; PP, peristaltic pump; CL, communication line; C_1 and C_2, microcolumn positions (reprinted from ref. [II] by courtesy of Elsevier).

The SI-BI-LOV method for handling of chelating Sepharose beads involves several different operational stages, namely, system preconditioning, beads loading and cleansing, sample loading for measurand sorption onto the beads, elution and beads disposal. The operating procedure of the method is described in detail in the paper [II] which is listed in the Appendix and summarized as follows:

A well defined small volume of beads suspension (20 μL) is aspirated from the beads reservoir and are withheld in position C_1 of the LOV. Following the delivery of diluted nitric acid (from the eluent container) and carrier to port 4, the beads are transported from column position C_1 to C_2 and simultaneously cleansed. Next, a well defined volume of sample is aspirated from port 5a and stored in HC. Subsequently it is mixed with the pH adjusting solution from SP2 and passes through column C_2, where the target ions are retained on the beads, while the matrix solution goes to the waste. Afterwards, a small volume of eluent is aspirated from port 2 and subsequently delivered to the measurand loaded beads position with stopped-flow period of 5 s in order to effect complete elution. In the meantime, the ETAAS is activated and the tip of the autosampler arm is moved into the graphite tube. Thereafter, the eluate, sandwiched by air segments, is transported into the graphite tube and the ETAAS instrument continues to run the temperature program for quantification. Finally, the used beads together with some carrier is aspirated back to column position C_1 from port 4, and discarded to the waste via port 3.

Experimental parameters, including pyrolysis temperature and atomization temperature of ETAAS instrument for the detection of Cd, Pb, Ni, sample acidity and SI-LOV variables such as sample flow rate, eluent concentration and volume, and elution flow rate were optimized by a univariate approach. The optimized parameters are summarized in Table 4-1.

The sample acidity is a key factor in the process of chelation of metal ions. The adjustment of sample pH to the optimal value (pH 5.0) is readily effected by employing a second external syringe pump which online injects pH-adjustment agent (1 M ammonium acetate at pH 5.0) into sample.

Table 4-1. Experimental parameters for SI-LOV-ETAAS system using hydrophilic chelating Sepharose for the determination of Cd, Pb and Ni.

Pyrolysis temp./Ramp time+Holding time:	
Cd	350 °C/10 s + 30 s
Pb	400 °C/10 s + 30 s
Ni	1100 °C/10 s + 30 s
Atomization temp./time:	
Cd	1400 °C/2 s
Pb	1600 °C/3 s
Ni	2150 °C/5 s
Column cleansing	400 μL of 2M HNO_3
Sample acidity	pH 5.0
Sample volume	1800 μL
Sample acidity	pH 5.0
Sample loading flow rate (μL s^{-1})	50
Eluent	50μL of 2M HNO_3
Eluent flow rate (μL s^{-1})	10 + stop flow (5s)

Experimental results showed metal ions were quickly adsorbed on the surface of the beads and there was rather limited variation in the integrated absorbance of Cd, Pb and Ni as a function of sample flow rate between 5 and 100 μL s^{-1}. It has been reported that the Sepharose beads act 50 times faster than the Chelex-100 ones, and this can be explained by the difference in the hydrophobicities and the anchoring of the chelating group to the support [95]. Sorbents based on hydrophilic appear to be faster in adsorption of metal ions than those based on an organic polymer matrix in aqueous medium.

In a previous work in our group Sephadex C-25 was used for the collection of nickel exhibits very compressible, that is, they can be trapped and transferred only at a low flow rate of less than 20 μL s^{-1} and at higher flow rate they become squeezed and can flow through the narrow space between the channel and the PEEK tubing stoppers [70]. On the other hand, the base matrix of Sepharose is rigid and permits the use of very high flow velocities. Finally as a practical option, a sample flow rate of 50 μL s^{-1} was employed.

4.3 Performance

In Table 4-2 is shown the analytical performance data for the system. As can be seen, chelating Sepharose features very high retention efficiency for Cd, Pb and Ni.

The accuracy of the method was evaluated by the analysis of biological reference materials, that is, CRM 320 (River sediment) and BCR No 279 (Sea lettuce) from The Community Bureau of Reference (BCR) and SRM 1640 (Natural water) from The National Institute of Standards & Technology (NIST). The experimental results (Table 4-3) obtained for those three standard materials agreed very well with the certified values.

Table 4-2. Analytical performance of the SI-LOV system using chelating Sepharose for the determination of Cd, Pb and Ni by ETAAS.

Parameter	**Cd**	**Pb**	**Ni**
Regression equation (AA=)	6.6003[Cd](μg L^{-1}) + 0.0173	0.1561[Pb](μg L^{-1}) + 0.0198	0.2917[Ni](μg L^{-1}) -0.0066
Linear range (μg L^{-1})	0.005-0.050	0.10-2.00	0.05-1.00
Bead volume (μl)	20	20	20
Sample frequency (h^{-1})	12	12	12
Retention efficiency (%)	95	75	90
Enrichment factor*	86	68	81
D.L. (μg L^{-1}) (n=11)	0.001	0.07	0.02
Precision (n=11)	3.6%	5.1%	3.2%

Table 4-3. Determination of Cd, Pb and Ni in certified reference materials.

Sample	**Measurand**	**Certified value**	**Found value (n=4)**	**Recovery (%)**
CRM279 (μg g^{-1})	Cd	0.274 ± 0.022	0.27 ±0.01	98
	Pb	13.48 ± 0.36	14.8±0.6	110
CRM320 (μg g^{-1})	Cd	0.533 ± 0.026	0.54±0.02	102
	Pb	42.3 ± 1.6	43±3	101
	Ni	75.2 ± 1.4	76±2	101
SRM1640 (μg kg^{-1})	Cd	22.79 ± 0.96	22.6+0.9	99
	Pb	27.89 ± 0.14	30±1	107
	Ni	27.4 +0.8	26.1±0.9	95

5. SI-BI-LOV-ETAAS schemes utilizing hydrophobic surfaces for the determination of metal ions [I, IV, VI, VII]

5.1 SI-BI-LOV-ETAAS schemes using PTFE beads as hydrophobic surfaces [I]

5.1.1 Introduction

Although a hydrophilic sorbent, such as chelating Sepharose, offers advantages such as trouble-free automatic handling within LOV-microconduit systems, high retention efficiencies and is applicable for a wide range of transitional metal elements, the lack of selectivity makes its ability to retain a given trace metal can become impaired by the lack of exchange capacity as a consequence of the saturation of the resin with the dominantly present interfering ions.

In comparison with hydrophilic bead materials, hydrophobic beads, which implicitly can retain only non-charged compounds, are potentially much more versatile and interesting. Enhanced selectivity can be achieved by intelligent selection of the chelating reagent via generation of the non-charged complex to be adsorbed on the surface of the hydrophobic surface.

The feasibility of the bead-injection/elution SI-LOV approach for handling hydrophobic Aldrich PTFE beads as a renewable column material for separation and preconcentration of trace metal ions by ETAAS was assessed, and it showed that PTFE beads offer much better performance as compared with the other hydrophobic material (C_{18}-PS/DVB) [68].

However, the inherent characteristics of the PTFE beads make it difficult to manipulate them in an on-line system. Bearing in mind the physical properties desired of the bead materials, such as morphology and density, the PTFE beads used appeared

far from ideal. Thus, under a microscope they emerged more like lumps of irregular shape, and were (despite initial sieving) of very non-uniform size. In operation, this led to progressively tighter packing of the columns, resulting in increased flow resistance together with incomplete transport of the loaded beads from one microcolumn to the other within the LOV. Since the density of PTFE (viz., 2.1 g mL^{-1}) is much higher than that of the suspending solvent used (ethanol), the beads tended to settle down quickly which, in turn, caused poorer reproducibility for manipulating and transferring them within the microconduit channels. Therefore, attempts were made to locate PTFE beads of perfect spherical shape and size homogeneity in order to exploit them in the LOV approach.

After a thorough search, a new kind of PTFE material, named Granular PTFE Algoflon®, came up, which, although not entirely ideal for our purposes, might serve as a potentially serious candidate, because it appeared to have the right morphology. Since it is spherical and possesses higher hydrophobicity than the previously used PTFE beads material, improvements in both the physical and the analytical performances were to be expected.

Therefore, the performance of the granular PTFE beads was examined and discussed by applying it in both SI and SI-LOV systems for the determination of trace amount of cadmium in environmental samples with complex matrices. Cadmium was selected as the model measurand because it is known to be a highly toxic metal element, which plays important roles in the biological metabolism.

5.1.2 Approach I: SI-BI-LOV scheme using PTFE beads in a renewable fashion

The diagram of the SI-LOV-ETAAS system used is schematically shown in Fig. 5-1.

An operational sequence, comprising system preconditioning, beads loading, sample derivatization/sorption, cleansing, elution, and beads discarding is described in detail in the paper [I]. In the proposed method, the sample, driven by SP_1, is merged with DDPA solution provided by SP_2, and the mixed solution is passed through the beads to the waste while the on-line formed Cd-DDPA chelate is retained on the

surface of the beads. Thereafter, a metered volume of ethanol is used for elution.

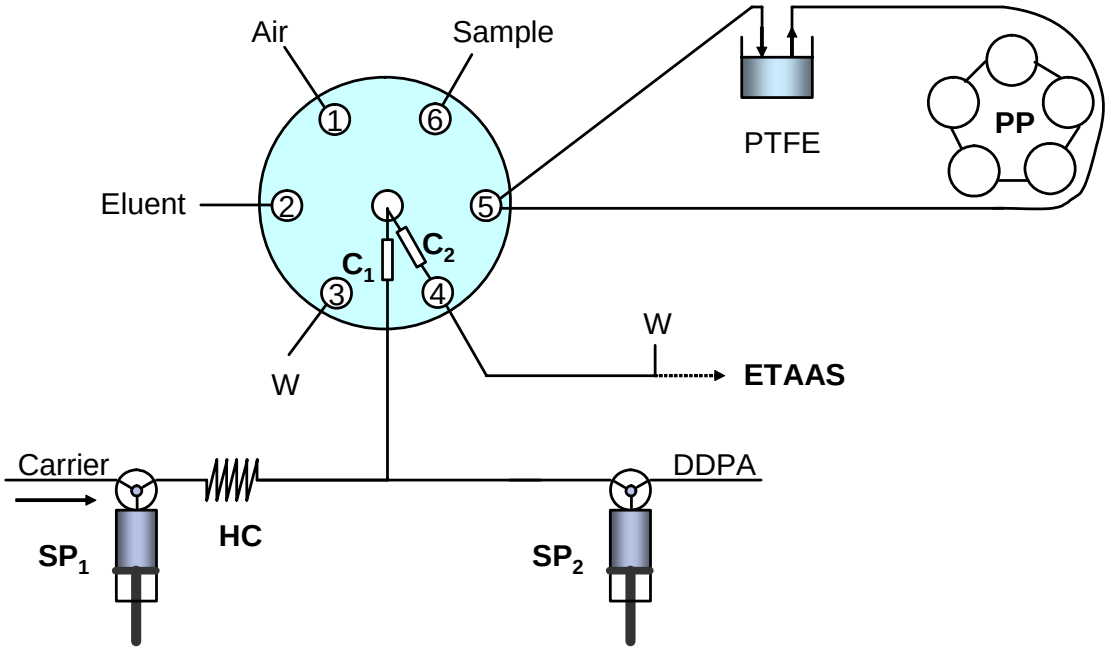


Fig. 5-1. Schematic diagram of the SI-LOV system for ETAAS detection of ultra-trace levels of cadmium using PTFE beads. SP_1 and SP_2, syringe pumps; C_1 and C_2, microcolumns; PP, peristaltic pump; PTFE, poly(tetrafluoroethylene) bead suspension; Eluent, mixture of ethanol and 0.5 % Triton X-100; Carrier, 0.05 % HNO_3; W, waste; DDPA (0.8% w/v) (reprinted from ref. [I] with courtesy of Elsevier).

Due to the hydrophobic nature and the high density of the PTFE beads, organic solvents containing absolute ethanol and Triton X-100 (0.5% v/v) and a magnetic bar stirrer were employed to form a stable and homogeneous PTFE beads suspension for reproducible manipulation within the LOV system.

The preliminary experimental results showed that the granular PTFE beads exhibited higher sensitivity when compared with the Aldrich beads.

However, the morphology of the granular PTFE bead underwent change in the long run, the beads breaking into smaller particles, which tended to pack and get adhered both within the circulating system and the LOV microcolumns. This can very likely be ascribed to a "memory effect", because the commercial Algoflon® beads, which are intended for coating purposes, actually are produced on the basis of small particles (25 μm), which through a manufacturing process are made into a product of

different beads sizes with a nominal diameter of 650 μm. When subjected to mechanical stimulus, they are obviously broken down to their original entities. So difficulties in circulating, aspirating and discarding the granular PTFE beads were not infrequently encountered.

In order to evaluate the analytical characteristics and performance of the granular PTFE beads it was, therefore, decided to examine this material in a sequential injection system furnished with permanent packed column, and then evaluate the results in comparison with those obtained earlier with the Aldrich PTFE beads in an SI-LOV system.

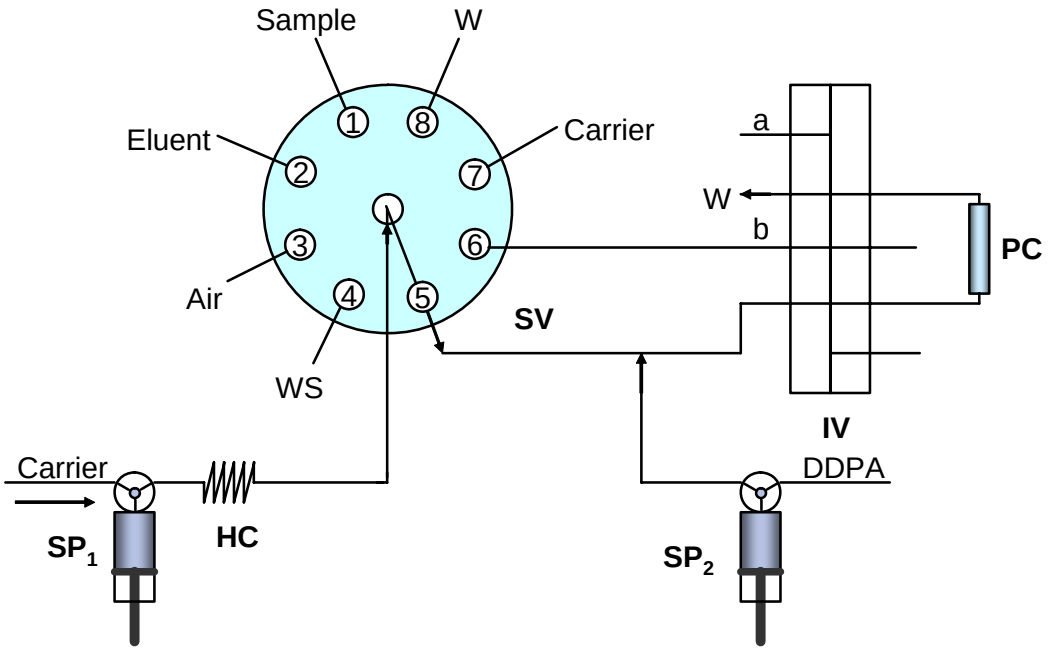
5.1.3 Approach II: SI scheme using PTFE beads in a permanently packed column mode

To evaluate the analytical performance of the granular PTFE beads and the Aldrich ones under the comparable conditions, a small packed column which imitates the amount of beads in the SI-LOV system was employed. Thus, 5 mg of the granular PTFE bead was used in the packed column, which is equal to the beads of the 60 μL of PTFE bead suspension used in the LOV microcolumn. The diagram of the SI-ETAAS system used is schematically shown in Fig. 5-2.

In operation, the injection valve is firstly set in the load position. Thus, Cd-DDPA chelate, which is generated on-line when the sample solution is mixed with the DDPA solution from SP_2, is retained on the sorbent in the packed column. Thereafter, the injection valve is changed to the inject position and a minute, well-defined volume of eluent from port 2 is aspirated for the elution of the column. The eluate is sandwiched by air segments and transported to ETAAS for quantification.

Parameters concerning ETAAS pyrolysis temperature and atomization temperature, the concentration of DDPA and sample acidity, and SI variables such as sample loading flow rate, eluent volume and elution flow rate were optimized. The optimized experimental conditions for SI system and ETAAS parameters are summarized in Table 5-1.

(a) Preconcentration (Load)



(b) Elution (Inject)

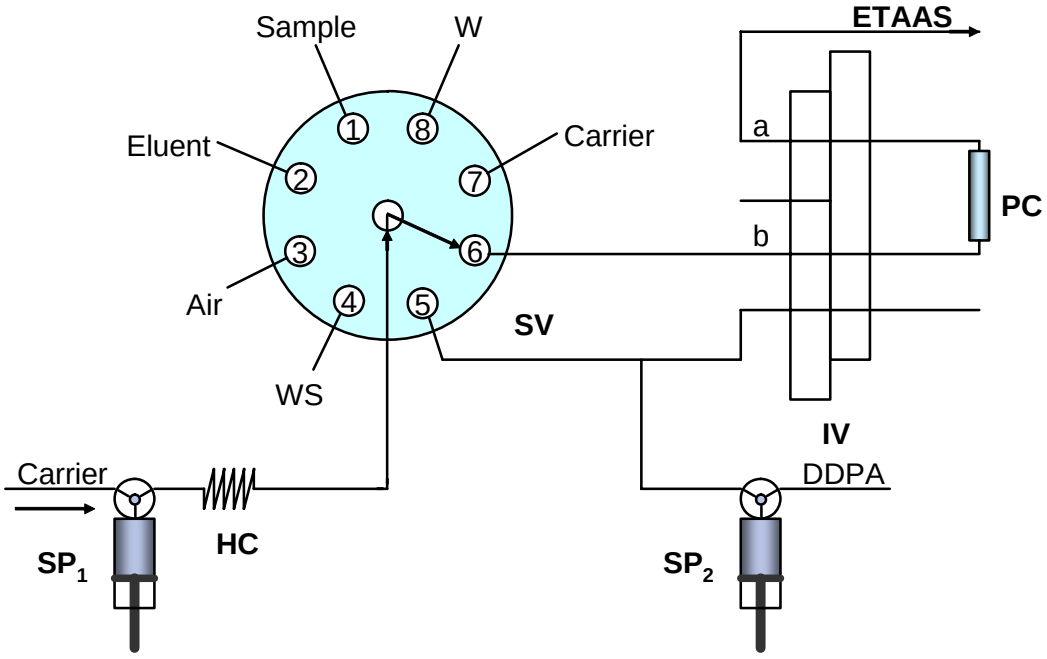


Fig. 5-2. Schematic diagram of the SI on-line column separation/preconcentration system with detection by ETAAS: a) load position; b) inject position. SP_1 and SP_2, syringe pumps; SV, 8–port selection valve; PC, packed column; HC, holding coil; IV, 2-position injection valve; Eluent, ethanol; Carrier solution (0.05 % HNO_3); WS, washing solution (0.2 % (w/v) DDPA); W, waste; DDPA (0.8 % w/v) (reprinted from ref. [I] with courtesy of Elsevier).

Table 5-1. Experimental parameters for SI-ETAAS system using granular PTFE beads for the determination of Cd.

Pyrolysis temp./Ramp time+Holding time	350 °C/15 s + 35 s
Atomization temp./time	1400 °C/5 s
DDPA conc. (w/v)	0.8%
Sorbent	Granular Algoflon® S131 (PTFE) beads
Sample acidity	2.0% HNO_3
Sample loading flow rate (μL s^{-1})	24
DDPA flow rate (μL s^{-1})	12
Eluent	50μL of ethanol
Flow rate of elution (μL s^{-1})	7

5.1.4 Performance

The analytical figures of merits of the packed column in the SI system using the granular PTFE beads as compared to the renewable microcolumn in the LOV system with Aldrich PTFE beads are listed in Table 5-2, including statistical parameters, sample throughput, sample and beads consumption, retention efficiency and enrichment factor.

Table 5-2. Analytical performance of the external packed column using the granular PTFE bead in the SI system as compared to the renewable microcolumn with Aldrich PTFE bead in the LOV system for the determination of Cd by ETAAS.

Parameter	**Granular PTFE bead**	**Aldrich PTFE bead**
Regression equation (n=5)	AA = 1.6107[Cd](μg L^{-1}) + 0.0040	AA = 0.2956[Cd](μg L^{-1}) + 0.0017
Linear calibration range (μg L^{-1})	0.05-0.25	0.05-1.00
Detection limit (ng L^{-1}) ($3\sigma_{blank}$, n=6)	5.5	5.0
Repeatability (%) (0.1 μg l^{-1}, n=6)	1.3	3.0
Reproducibility (%) (0.1 μg L^{-1}, n=4)	1.0	4.3
Bead consumption	5 mg (equal to 60 μL)	60 μL
Sample volume (μL)	1250	1250
Sample frequency (h^{-1})	12	12
Retention efficiency (%)	82	74
Enrichment factor	20.6	17.2

As seen from the table, the granular PTFE beads exhibit much improved sensitivity, that is, from 0.2956 to 1.6107 in comparison with the Aldrich beads. The

packed column yields much better repeatability and reproducibility values. The enrichment factor and retention efficiency become improved from 17.2 to 20.6 and 74% to 82%, respectively.

In the packed column, any difficulties of transport and discarding beads were avoided and the beads can be used repeatedly and disposed intermittently.

When columns of larger volumes were prepared, clogging problems started to arise at high flow rates or over extended time of use if more than 75 mg of beads were packed. However, when using the small columns with 5 mg of beads the function was entirely trouble-free: No backpressure effects nor any tendency of packing was observed even after long term uni-directional operation. Besides, eluting the column completely with a well-defined, small volume of eluate was readily feasible.

Therefore, from an analytical point of view, the improvements in the operational characteristics reveal the granular PTFE beads to constitute a more potent and promising material for implementing separation and preconcentration than the Aldrich PTFE beads.

The proposed approach employing the granular PTFE beads packed column was applied to the determination of trace levels of cadmium in three certified reference materials: CRM 279 (Sea Lettuce), CRM 320 (River Sediment) and SRM 1640 (Natural Water). The experimental results showed that the concentrations of cadmium determined were in good agreement with the certified reference values, with percentage recoveries of 99, 96 and 97 for CRM 279, CRM 320 and SRM 1640, respectively (Table 5-3).

Table 5-3. Determination of cadmium in certified reference materials using the granular PTFE bead in the SI system and ETAAS detection (ref. [I]).

Sample	Certified value ($\mu g\ g^{-1}$)	Found value (n=4)($\mu g\ g^{-1}$)	Recovery (%)
CRM279	0.274 ± 0.022	0.273 ± 0.013	99
CRM320	0.533 ± 0.026	0.516 ± 0.006	96
SRM1640	22.79 ± 0.96	22.30 ± 1.13	97

5.2 SI-BI-LOV-ETAAS using renewable hydrophobic bead surfaces with immobilised chelating agent [IV, VI]

5.2.1 Introduction

One of the most common approaches for solid phase extraction of target metal ions via hydrophobic interactions on reversed-phase materials is to mix the target metal ions with the chelating reagent on-line to form the neutral chelates, which are subsequently retained on the sorbent-filled column reactor, as described in Chapter 5.1. This approach has been successfully applied in many instances, but it might not be applicable when relatively slow kinetics of the retention of the resulting chelate on the bead surface is of significance.

The solution to that problem can be solved by resorting to an approach entailing off-line preimpregnation of the hydrophobic beads with the selected ligand. The obvious advantage is that the immobilization conditions, including the pH-value and the time, can be optimized to ensure adequate sorption of the organic ligand onto the hydrophobic surfaces. The kinetic problems related to adsorption of chelate can hereby be vastly reduced or even eliminated.

Furthermore, a significant advantage of using the bead-renewable approach in the LOV configuration can also be obtained in the elution step. Thus, it does not matter how the chelate actually is stripped from the hydrophobic surface: whether it involves the release of the whole complex, or a splitting up of the complex (where the ligand might either remain on the bead surface or dissolve into the eluent medium), or as a combination of both, is of no concern, because the beads are readily renewed in each measurement cycle.

This concept is demonstrated by taking as example the determination of Cr(VI) employing hydrophobic beads consisting of poly(styrene-divinylbenzene) containing pendant octadecyl moities (C_{18}-PS/DVB) which are preimpregnated off-line with 1,5-diphenylcarbazide (DPC). Although the determination of Cr(VI) with DPC is a

well-known and widely used procedure in batch assays, it was found virtually impossible to implement the chemistry on-line with the naked hydrophobic beads. This is due to the very slow process in the adsorption of the ligand onto the bead surface. Therefore, it is evident that the use of preimpregnated beads in the LOV microconduits is particularly advantageous for this application.

5.2.2 Method development: Configuration, parameters and operational procedure description

The derivatization reaction between Cr(VI) and DPC shown below contains a two-step process involving the oxidation of carbazide (H_4L), affixed on the preimpregnated beads, to carbazone (H_2L), and final chelation of the oxidized reagent with the generated half of the Cr(III) species, and hence retained on the beads, while the other half is wasted:

$$2CrO_4^{2-} + 3H_4L + 8H^+ \longrightarrow 2[Cr(VI)\text{-}H_4L]$$

(H_4L: beads; $2[Cr(VI)\text{-}H_4L]$: beads)

$$2[Cr(VI)\text{-}H_4L] \longrightarrow [Cr(HL)_2]^+ + Cr^{3+} + H_2L + 8H_2O$$

($[Cr(HL)_2]^+$: beads)

Prior to running the SI-BI-LOV method, the C_{18}-PS/DVB beads are firstly cleansed by methanol and vacuum-dried. Then the beads are exposed to DPC solution for beads modification. Thereafter, the DPC-loaded beads are aspirated into a 1-mL plastic syringe for further use. The flow network for the actual analytical procedure is schematically illustrated in Fig. 5-3. The detailed operational stages of the SI-BI-LOV method, involving system preconditioning, sample and bead loading and preconcentration, elution, bead discarding, is listed in the paper [IV] in Appendix 2.

The experimental parameters, involving chemical and physical ones, were investigated. The reaction between DPC with Cr(VI) is favoured at high sample acidities [96], but sample acidification can bias the analytical results in natural waters as a consequence of progressive reduction of the Cr(VI) to Cr(III) by dissolved

organic matter [97,98]. In order to avoid the shift of the natural equilibrium between oxidation states, an external syringe pump for delivery of acid is used. Thus, the untreated sample zone is made to merge synchronously with a defined volume of acid solution delivered via the ancillary syringe pump. Hence, automated on-line pH adjustment of the sample plug is attained immediately prior to its reaching the DPC-coated beads, as loaded in the LOV unit, entailing minimum alteration of the original distribution of species in the sample. The effect of the concentration of nitric acid for pH adjustment was investigated. The enrichment of the LOV-packed beads decreases sharply for concentrations of ≤ 0.5 mol L^{-1} H^+. Therefore, a 5 mol L^{-1} HNO_3 solution that yields a final acidity of 1.0 mol L^{-1} H^+ for the solid-phase derivatization reaction was selected.

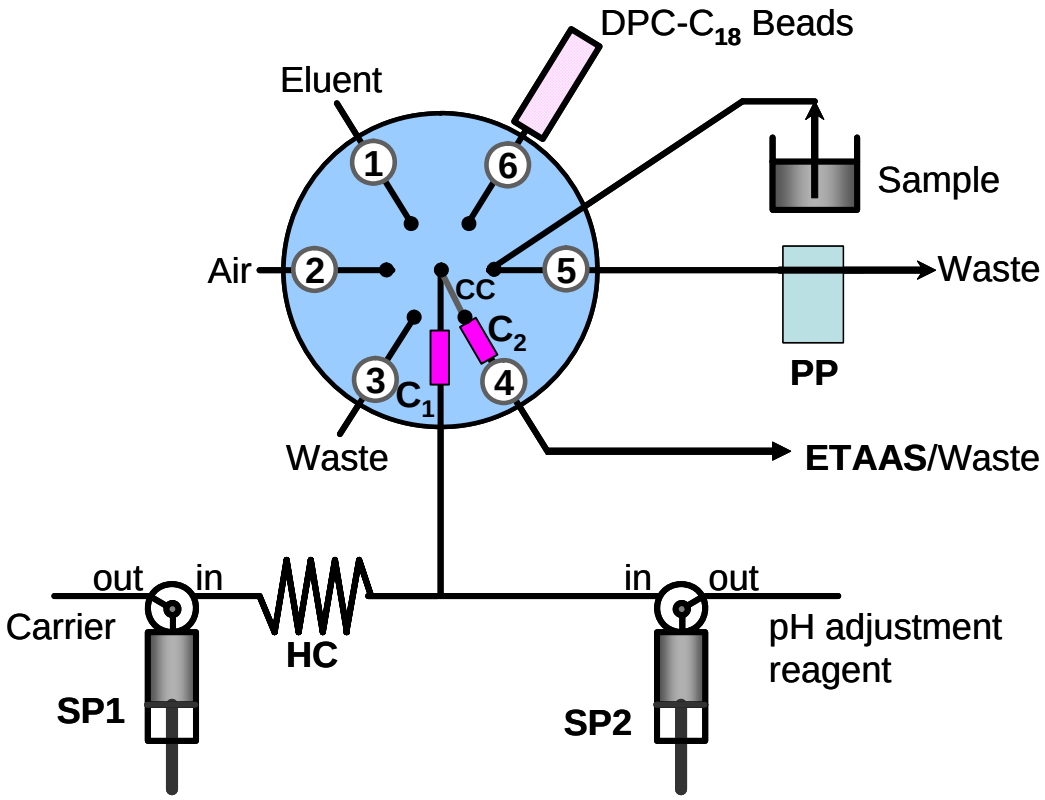


Fig. 5-3. Schematic illustration of the SI-BI-LOV flow network handling DPC-loaded C_{18}-PS-DVB renewable surfaces for automatic preconcentration and determination of traces of Cr(VI) via ETAAS detection. Carrier, 0.5 mol L^{-1} HNO_3; pH adjustment reagent, 5 mol L^{-1} HNO_3; SP, Syringe pump; PP, Peristaltic pump; C_1 and C_2, LOV microcolumn positions; PP, Peristaltic pump; HC, Holding Coil; CC, Central communication conduit; ETAAS, Electrothermal Atomic Absorption Spectrometry. DPC:1,5-diphenylcarbazide (reprinted from ref. [IV] with courtesy of American Chemical Society).

The preparation of the DPC-carrying particles prior to the SI-LOV sample pre-treatment was found to be crucial for successful performance of the automated approach in terms of retention capability for Cr(VI) and manipulation of the sorptive surfaces. The effect of the DPC concentration on the analytical features of the physically modified beads was evaluated from 0.0072 to 0.114% (v/v). Although experimental results revealed that concentrations of 0.036% DPC (v/v) and onward rendered constant analytical sensitivity, the highest concentration of DPC (i.e., 0.144%) was selected due to the slight increase in the size of the entities which facilitates their manipulation within the microconduit system.

Experimental results showed that mineral acid such as nitric acid with a concentration as high as 5 mol L^{-1} HNO_3 released less than 30% of the retained measurand. On the contrary, as a consequence of irreversible sorption, 90% (v/v) methanol quantitatively strips the immobilized Cr-H_2L chelate from the beads. Concomitant removal of the immobilized DPC occurs, thereby precluding the repeated use of the beads. Yet, the renewable sorbent scheme for each analytical cycle is a unique solution of that.

Other parameters such as sample loading flow rate, eluent volume and elution flow rate were also optimized. The optimized experimental conditions for SI-LOV system and ETAAS parameters are summarized in Table 5-4.

Table 5-4. Experimental parameters for SI-LOV-ETAAS system using DPC-impregnated C_{18}-PS/DVB entities for the determination of Cr(VI).

Pyrolysis temp./Ramp time+Holding time	1500 °C/10 s + 20 s
Atomization temp./time	2300 °C/4 s
DPC conc. (v/v) for beads preimpregnation	0.144%
Sorbent	DPC-loaded C_{18}-PS/DVB beads
Sample acidity	1 mol L^{-1} HNO_3
Total loading flow rate (sample + acid) (μL s^{-1})	75
Eluent	30μL of 90% methanol
Flow rate of elution (μL s^{-1})	4

5.2.3 Performance

In Table 5-5 is shown the analytical performance of the developed procedure for Cr(VI) determination by exploiting hydrophobic beads with physically immobilised DPC. As seen from the table, it features a very low limit of detection and excellent repeatability for the renewable sorbent approach.

Table 5-5. Analytical performance for the SI-LOV system using DPC-loaded C_{18}-PS/DVB beads with ensuing determination by ETAAS.

Regression equation (Cr, μg L^{-1})	0.2691[Cr] + 0.0022
Linear range (μg L^{-1})	0.12-1.5
Sample volume (mL)	2
Sample frequency (h^{-1})	15
Retention efficiency (%)*	38.8
Enrichment factor	12.9
Detection limit (μg L^{-1}; 3σ)	0.03
Precision (%)	3.8

*: This value theoretically can not exceed 50%.

The procedure wasa applied for the determination of total chromium in an NIST standard reference material (NIST 1640, natural water) after Cr(III) oxidation and Cr(VI) in other environmental waters. The results obtained are listed in Table 5-6. The results are in good agreement with the certified value and satisfactory recoveries were obtained in natural waters spiked with three levels of Cr(VI).

This project has been used in the study of a Ph.D. course named *Measurement Uncertainty in Chemical Analysis* for the calculation of the uncertainty of the measurand. The detailed procedure for the preparation of the uncertainty budget is contained in the Appendix of this thesis.

Table 5-6. Determination of trace levels of hexavalent chromium in environmental waters by on-line hyphenation of SI-BI-LOV using DPC-impregnated C_{18}-PS/DVB entities with ETAAS.

Sample	Added (μg L^{-1})	Found (μg L^{-1})[b]	Recovery (%)
Natural Water	---	< LOD	---
(SRM 1640)[a]	0.22	0.21±0.01	95.4
	0.45	0.47±0.02	104.4
	0.67	0.69±0.02	103.0
Tap water	---	0.040±0.002	---
(Hard water)	0.22	0.25±0.02	96.2
	0.45	0.50±0.04	102.0
	0.67	0.74±0.04	104.2
Seawater	---	0.108±0.009	---
	0.22	0.33±0.02	100.6
	0.36	0.45±0.03	96.2
	0.49	0.63±0.04	105.3

[a] Certified concentration of total chromium: 38.6± 1.6μg L^{-1}. Dilution factor, 1:100

[b]Results are expressed as the mean of 3 replicates ± standard deviation

5.3 SI-BI-LOV scheme using sorbent containing a balanced ratio of hydrophilic and lipophilic monomers [VII]

5.3.1 Introduction

Among the two categories of sorbents employed in SI-LOV system with SPE, the lack of selectivity of hydrophilic sorbents limits their application for samples containing large amounts of alkaline earth elements, although they offer various advantages such as trouble-free handling within the microconduit systems and are applicable for a wide range of transitional metal elements.

On the contrary, hydrophobic sorbents such as PTFE and C_{18}-silicagel, using for uptake of non-charged chelates generated by reaction of the measurand with an appropriate, ideally selective, complexing reagent, exhibit advantage in the potentially high selectivity. However, a problem is to reconcile the disagreement of the

hydrophobic properties of the beads with the aqueous media.

Therefore, a kind of sorbent which not only possesses capacity for hydrophobic interaction for the collection of neutral chelate of the measruand and chelating reagent, but also at the meantime offers hydrophilic properties which benefit its handling in the microconduit of the LOV unit, is expected to be an ideal one for SI-LOV system with SPE. Among various beads available, Oasis® HLB (*viz.*, poly(divinylbenzene-co-N-vinylpyrrolidone) is a lipophilic/hydrophilic copolymeric sorbent, which, as opposed to conventional hydrophobic sorbents such as PTFE and C_{18}-silicagel [68,70,99], not only possesses a superior reversed-phase retention capacity for non-ionic and moderately polar species, but also has physical features such as perfect spherical bead shape and uniform particle size distribution. The commercially available sorbent has an average dry particle size 30 μm, and the water-wettable beads ca be readily captured in the LOV micro-column cavities. Thus, it was selected as a sorptive material for SPE in a SI-LOV system for the determination of nickel. The determination of nickel has drawn increased interest, partly because some of its compounds are carcinogenic [94,100]. It is well known that Ni(II) and DMG share a very characteristic, selective reaction in which a neutral coordination compound ($Ni(DMG)_2$) is produced.

5.3.2 Method development: Configuration, parameters and operational procedure description

The SI-LOV-ETAAS system is depicted in Fig 5-4. The sequence of an analytical cycle comprising system preconditioning, sorbent loading, sample aspiration into the HC and mixing with DMG solution, loading of the generated compound ($Ni(DMG)_2$) onto the beads for separation and preconcentration, elution and finally beads disposal is described in detail in the paper [VII] in the Appendix.

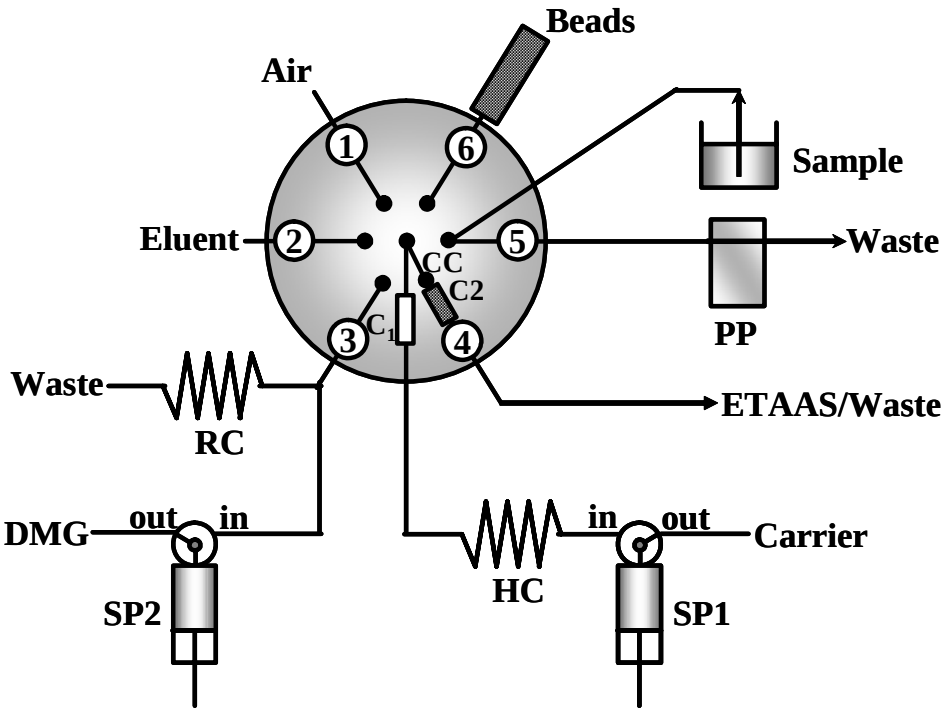


Fig. 5-4. Schematic diagram of the SI-LOV-ETAAS system for on-line determination of Ni(II) via chelation with DMG and preconcentration on poly(divinylbenzene-co-N-vinylpyrrolidone) beads. Carrier, 0.2 mol L^{-1} ammonium citrate buffer (pH 9.0); DMG, 1.2% (w/v) dimethylglyoxime in ethanol; Eluent, methanol; SP1 and SP2, Syringe pumps 1 and 2; C_1 and C_2, LOV micro-column positions; HC, Holding coil; RC, reaction coil; CC, Central communication conduit; PP, Peristaltic pump, ETAAS, electrothermal atomic absorption spectrometer (reprinted from ref. [VII] by courtesy of Springer-Verlag).

The aforementioned approach, described in Chapter 5.1, utilizing hydrophobic sorbents to collect on-line generated neutral compounds is not directly applicable due to slow kinetics in the generation of the non-charged chelates of Ni(II) and DMG solution. Thus, simple on-line mixing of the reactants did not result in any retention of Ni(II), indicating that the formation of the $Ni(DMG)_2$ chelate was slow and therefore a delay time for full reaction had to be incorporated prior to adsorption. To facilitate the development of the reaction for the on-line formation of the sparingly water-soluble $Ni(DMG)_2$ chelate, an auxiliary reaction coil mounted to one of the peripheral ports of the selection valve is employed. Hence, the mixture of sample and complexing reagent might remain there at will by appropriate flow programming to acquire sufficient reaction time prior to the application of backward-flow for the collection of the generated precipitate onto the microcolumn. After the investigation

of different residence time ranging from 70 to 250s, it was found that 70s of reaction time was sufficient for the on-line generation of the $Ni(DMG)_2$.

The complexation reaction between nickel and DMG involves the generation of oxonium ions, whereby the formation of the nickel chelate is favored under a buffered alkaline medium. Hence, the effect of pH on the yield of $Ni(DMG)_2$ formation was investigated. The experimental results showed that the analytical performance is optimal at pH 9.0. An ammonium buffer (pH 9.0) was used for pH adjustment. However, a surplus of ammonia is inappropriate because the competitive complexation reaction of Ni(II) with ammonia might increase the solubility of $Ni(DMG)_2$. As a compromise between the above mentioned factors, a buffer concentration of 0.2 mol L^{-1} was selected for the remainder of the studies.

To prevent the formation of insoluble oxyhydroxides of metal ions, such as Cr(III) and Fe(III), in real-life samples, citrate was introduced to selectively form tightly bound soluble complexes with these metals. Ammonium citrate can thus not only serve as buffer, but also as a masking reagent for potentially interfering species. Therefore, a 0.2 mol L^{-1} ammonium citrate buffer (pH 9.0) was employed for preparation of the entire set of samples and working standards.

Parameters concerning chelating reagent concentration, sample loading flow rate, eluent volume and elution flow rate were examined. Chemical variables and the optimized experimental conditions for SI-LOV system and ETAAS parameters are summarized in Table 5-7.

Table 5-7. Experimental parameters for SI-LOV-ETAAS system exploiting poly(divinylbenzene-co-N-vinylpyrrolidone) beads for the determination of trace level concentrations of nickel.

Pyrolysis temp./Ramp time+Holding time	1100 °C/10 s + 30 s
Atomization temp./time	2150 °C/5 s
DMG conc. In ethanol (w/v)	1.2 %
Sorbent	Poly(divinylbenzene-co-N-vinylpyrrolidone)
Sample acidity	pH 9.0
Sample loading flow rate (μL s^{-1})	50
Eluent	50μL of MeOH
Flow rate of elution (μL s^{-1})	10 + stopped flow (5 s)

5.3.3 Performance

The performance of this hydrophilic/hydrophobic balanced sorbent was compared with that of another reversed phase sorbent, namely, C_{18}-PS/DVB, and it showed very similar performance in most of the listed items, except as to the sample loading flow rate and statistical parameters (Table 5-8). Thus, the C_{18}-PS/DVB material needs a relatively lower sample loading flow rate (20 μL s^{-1}) to assure a complete collection of the nickel precipitate as compared to the co-polymeric material Oasis® HLB does (*viz.*, 50 μL s^{-1}). Moreover, C_{18}-PS/DVB yields poorer reproducibility. Since all the microfluidic operations are conducted in an aqueous environment, the sorbent with more hydrophilic properties should take benefit in performance. The hydrophilic monomers of the co-polymeric material can greatly facilitate the interaction between the beads surface and the aqueous solution, thereby allowing a faster mass transport on the liquid-solid interface. The reason for the better reproducibility of the water-wettable sorbent should be attributed to the straightforward handling in the micro LOV channel system as compared with its counterpart.

Table 5-8. Analytical performance of the μSI-BI-LOV system exploiting Oasis® HLB beads (poly(divinylbenzene-co-N-vinylpyrrolidone)) for the determination of Ni(II) and its comparison with C_{18}-PS/DVB as a sorptive material.

Parameter	**C_{18}-PS/DVB**	**Oasis® HLB**
Regression equation	0.1969 [Ni, μg L^{-1}] +0.0698	0.2057 [Ni, μg L^{-1}] +0.0592
Linear range/μg L^{-1}	0.2 - 2	0.2 – 2
Sorbent volume/μL	20	20
Sample volume/mL	1.8	1.8
Eluent volume/μL	50	50
Loading flow rate/μL s^{-1}	20	50
Sample frequency/h^{-1}	8	10
Retention efficiency (%)	66	69
Enrichment factor	24	25
Detection limit/μg L^{-1} (3σ)	0.04	0.05
Repeatability (%)	4.5	4.8
Reproducibility (%)	7.2	5.6

The tolerance of the proposed method to the most commonly encountered ions in

environmental waters, such as Ca(II), Mg(II), Pb(II), Cu(II), Cd(II), Fe(III), Mn(II) and Co(II), was investigated. The experimental results showed that the interference level was < 10% when the ratio of interfering species, such as Ca(II) and Mg(II), to measurand was below 1×10^6, while a ratio of interferent to measurand below 1×10^4 for Pb(II), Cu(II), Cd(II), Fe(III), Mn(II) and Co(II) caused no significant deviations in Ni(II) recoveries. The high tolerance to alkaline earth elements implies the possibility of direct analysis of hard waters and seawater with no need for any sample pretreatment.

The experimental results of the proposed methodology applied for the determination of nickel in an NIST standard reference material (viz., NIST 1640 - Trace elements in natural water), household tap water of high hardness and local seawater are listed in Table 5-9. Satisfactory recoveries were achieved for all spiked environmental water samples with maximum deviations of 6%. The experimental results for the standard reference material (30.0±1.2 μg L^{-1}) were not statistically different to the certified value (27.4±0.8 μg L^{-1}) at a significance level of 0.05.

Table 5-9. Determination of trace levels of Ni(II) in environmental waters using the μSI-BI-LOV-ETAAS system with poly(divinylbenzene-co-N-vinylpyrrolidone) beads as sorptive material.

Sample	**Added/μg L^{-1}**	**Found/μg L^{-1}[b]**	**Recovery (%)**
Hard tap water[a]	---	0.21±0.01	---
(Kgs.Lyngby, Denmark)	0.50	0.70±0.04	98
	0.75	0.93±0.06	95
Seawater[a]	---	0.56±0.01	---
(Klampenborg, Denmark)	0.5	1.06±0.06	99
	0.1	1.50±0.04	94

[a] Dilution factor, 40:50

[b] The results are given as the mean of 3 replicates ± standard deviation

6. SI-BI-LOV-ETAAS schemes for on-line speciation analysis of trace levels of Cr(III) and Cr(VI) [III, VI]

6.1 Introduction

The growing awareness of the strong dependence of the toxicity and biological activity of the elements on their chemical forms or oxidation states has resulted in an increasing interest in chemical speciation of metal species in inorganic analysis in the past decades [101]. A great challenge for the analytical chemists is to develop appropriate analytical techniques for accurate quantitation of the individual species and ensuring appropriate sensitivity for environmental monitoring.

Taking advantage of being readily able to control the kinetic conditions, sequential injection (SI) has been explored for the application of chemical speciation, and the potentials of it for on-line chemical speciation have been recently summarized by van Staden and Stefan [102]. In this context, its further development, the LOV format, which provides an unprecedented versatility for on-line sample manipulations and readily can realize a particular analysis [103], is expected to be a promising tool for chemical speciation.

The potential of SI-LOV for chemical speciation is demonstrated for the measurement of the two main chromium oxidation states, that is, Cr(VI) and Cr(III). Chromium is commonly present in soils, waters, rocks, fauna and flora, and in volcanic dust and gases. The occurrence of chromium due to human activities results mainly from production of waste water through metallic smelting, electroplating, hide processing and dye stuff industries. The biological and chemical properties of the two species differ significantly. Trivalent chromium is an essential micronutrient in the diet of mammals to maintain effective glucose, lipid and protein metabolism [104]. In contrast, hexavalent chromium is highly toxic and carcinogenic for a variety of organisms as a result of its elevated oxidation potential and the ability to penetrate

biological membranes [105].

6.2 Method development: Configuration, parameters and operational procedure description

As stated earlier, hydrophilic chelating Sepharose has proven to constitute an ideal sorbent in LOV application with high retention efficiencies and fast adsorption rates for many transitional metal ions [II]. In slightly acidic media, Cr(III) exists as the cation Cr^{3+}, while Cr(VI) is presented in anionic form. Therefore, chelating Sepharose was chosen as a sorbent for SPE in LOV for the collection of Cr(III). In most flow-injection/continuous-flow chemical speciation works, off-line oxidation or reduction in the batch mode has often been employed to effect the chemical conversion of one species to the other one [106-108], but the risk of sample contamination is much higher and the procedure is labor-intensive. Therefore, on-line conversion of Cr(VI) to Cr(III) should prove a promising approach.

The operational procedure, described in detail in the paper [III] in the Appendix, is outlined as depicted in Fig. 6-1 and implemented in the LOV-manifold depicted in Fig. 6-2. The aspirated sample solution is initially divided into two portions for Cr(III) and total Cr (Cr(III) and Cr(VI)) measurements, respectively. The portion of aqueous sample solution for total Cr measurement is firstly aspirated into reaction coil (RC) to effect reduction from Cr(VI) to Cr(III). The reduction is a slow process, and even the reductant ultimately adopted (see below), it requires ca. 4 min to lead to a constant and reproducible conversion. However, this is perfectly compatible with the time required by the system to run through the entire procedure for the Cr(III) determination, including ETAAS determination of the second portion of sample. Therefore, during the process of reduction in the RC, another sample aliquot is aspirated for Cr(III) determination, that is, Cr(III) is retained on the beads and subsequently eluted and quantified. Thereafter, the reduced sample portion is

aspirated back to the HC and processed in the same way for total Cr. Thus, the sum of Cr(VI) and Cr(III), is determined. Hence, the Cr(VI) content is obtained by the difference between the concentration of Cr(III) and the sum of Cr(III) and Cr(VI).

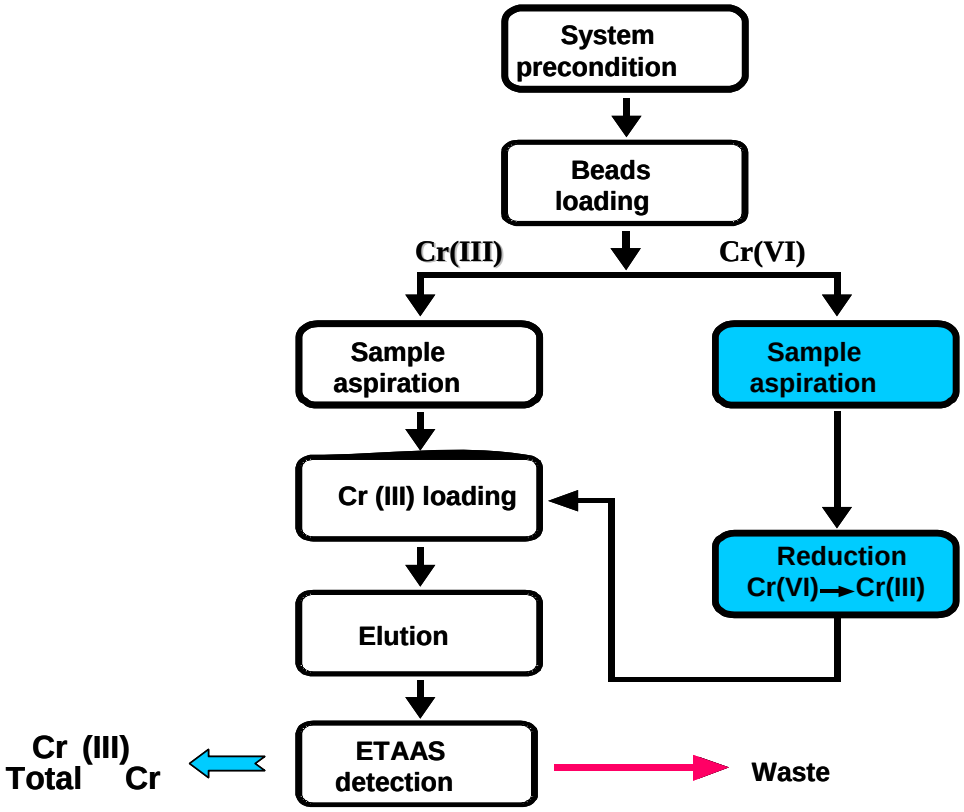


Fig. 6-1. Flow chart of the SI-procedures to which the Cr(III) and the Cr(VI) species, present in the original sample solution, are subjected. Thus, while the Cr(III) ions are separated/preconcentrated on the chelating Sepharose beads and subsequently eluted and quantified by ETAAS, the Cr(VI) ions are reduced to Cr(III) by hydroxylamine (in an open reaction coil as shown in Fig. 6-2), and afterwards treated as the native Cr(III) ions (reprinted from ref. [III] with courtesy of The Royal Society of Chemistry).

Different strong organic and inorganic reductants such as ferrous iron [109], potassium sulfite [110], hydroxylamine [111- 114], hydrogen peroxide [115,116], and ascorbic acid [117- 120], mainly in acidic medium, have been employed for Cr(VI) reduction. The effectiveness of these reductants for on-line conversion of Cr(VI) to Cr(III) at pH 4.0 is depicted in Fig. 6-3. Ascorbic acid has generally been regarded as the most ideal one in several publications due to its high reaction rates and improved reduction efficiency. However, it was not so effective in our system at

the 0.05 mol L^{-1} level with on-line reduction, or even in the off-line reduction mode. This is probably due to the fact that the former authors monitored the fading of the Cr(VI) solution spectrophotometrically, while in our SI-LOV system only the free or labile Cr(III) can be retained on the sorbent and therefore determined, and that an inert dehydroascorbic acid (DA) complex of Cr(III) is generated during the reduction process of Cr(VI) [112].

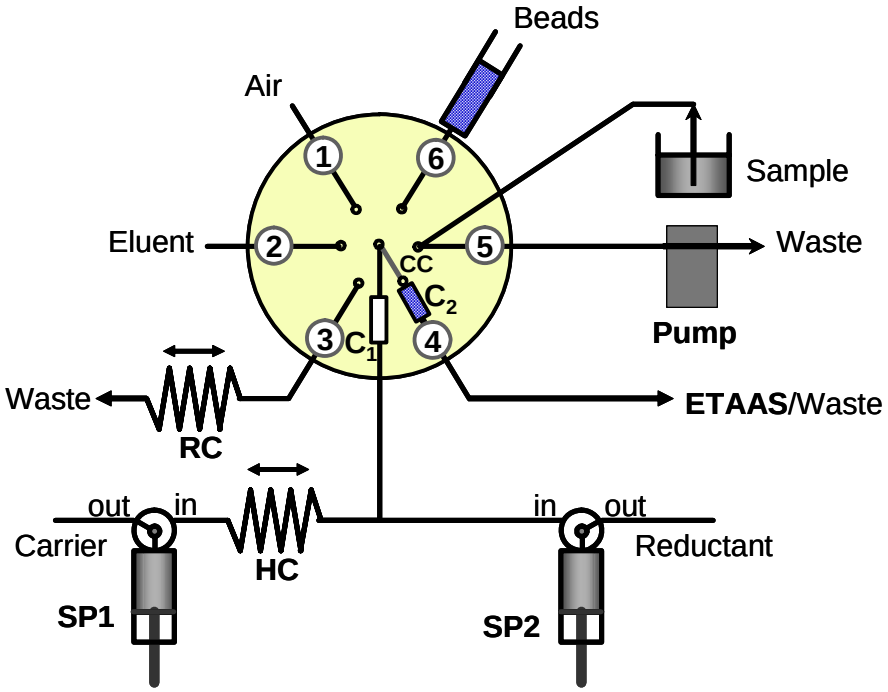


Fig. 6-2. Schematic drawing of the μSI-BI-LOV-ETAAS system for Cr(III) and Cr(VI) speciation. Carrier, 0.005 mol L^{-1} formic acid/formate buffer at pH 3.8; Reductant, 0.02 mol L^{-1} hydroxylamine in 0.005 mol L^{-1} pH 3.8 buffer; Eluent, 0.1 mol L^{-1} HNO_3; Beads, Chelating Sepharose; SP1/SP2, Syringe pumps 1 and 2; C_1 and C_2, LOV microcolumn positions; HC, Holding coil; RC, Reaction coil; CC, Central communication conduit; ETAAS, electrothermal atomic absorption spectrometric instrument (reprinted from ref. [III] with courtesy of The Royal Society of Chemistry).

Hydrogen peroxide in acidic medium (0.05 mol L^{-1}; pH = 4.0) was also not efficient in reduction and has poor reproducibility as a consequence of the vapor bubbles generated in the miniaturized system, which made the on-line aspiration and delivering of the reagent troublesome.

On the other hand, preliminary batch experiments confirmed that Cr(VI) was quantitatively reduced by hydroxylamine (0.05 mol L^{-1}; pH = 4.0) at room temperature without further disturbance of the sorption process. Optimization

experiment for the concentration of hydroxylamine for the automated Cr(VI) reduction was conducted and the results are shown in Fig. 6-4. As can be seen from the figure, the highest reduction efficiency is obtained around a concentration of 0.02 mol L^{-1} which was finally chosen. Lower concentrations did not expedite the redox reaction while higher concentrations gave rise to a higher reagent blank.

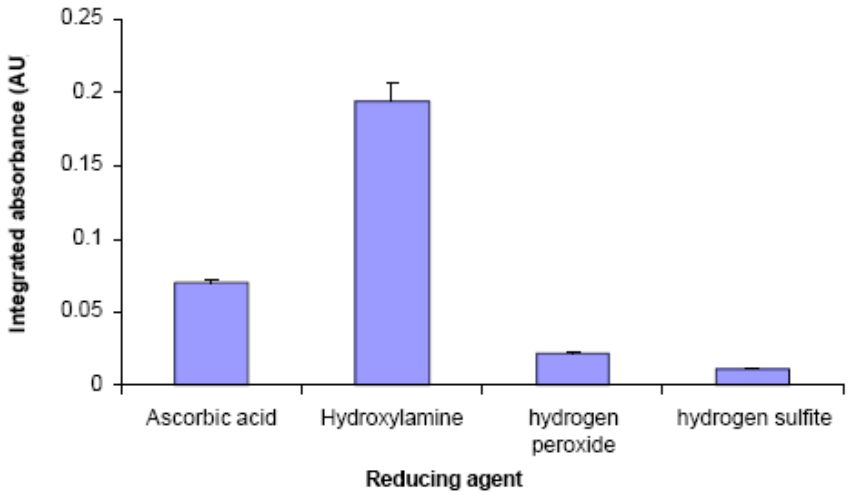


Fig. 6-3. Comparison of the effectiveness of different kinds of reducing reagents for on-line conversion of Cr(VI) into Cr(III) at pH 4.0. All the reducing agents are prepared at the 0.05 mol L^{-1} level. Sample loading volume: 1.8 mL; Sample to reductant volume ratio: 4:1; Eluent volume: 25 μL; Cr(VI) concentration: 0.4 μg L^{-1}; Reduction time: 180 s (reprinted from ref. [III] with courtesy of The Royal Society of Chemistry).

The pH of the reductant is important not only in respect to the reaction development rate, but also because the sorption of Cr(III) on the chelating surfaces is strongly depending on it. The more acidic the medium is, the more beneficial for the reduction. The widely used pH for quantitative conversion of Cr(VI) into Cr(III) by hydroxylamine is 2.0±0.2 [112-114]. Yet, the best retention yields for Cr(III) are attained above pH 3.5. At a lower pH the functional groups of the chelating Sepharose are protonated, thus hindering the sorptive preconcentration of Cr(III), while at milder acid or alkaline conditions hydrolysis of the metal ions occur, making them inaccessible for the sorptive material. So a compromise between the reduction of Cr(VI) and the chelation of Cr(III) must be made. Fig. 6-4 shows the analytical

signals recorded after on-line reduction and preconcentration of chromium species at different sample pH-values. As can be seen, the highest sensitivity is obtained within the pH range of 3.5-4.0. Therefore, the reducing agent and carrier solution were adjusted to a final pH of 3.8. For the samples, this pH adjustment was done immediately before injection into the flow set-up to minimize re-distribution between oxidation states.

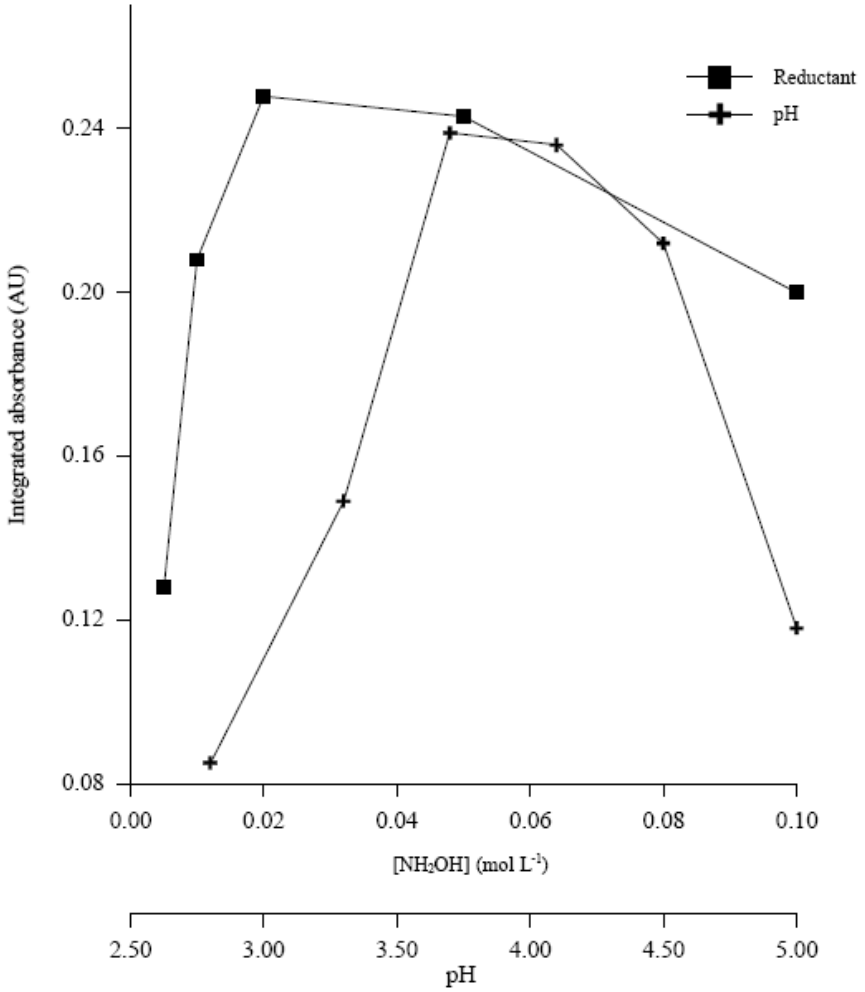


Fig. 4. Effect of the nominal concentration of hydroxylamine (■) and pH (▲) on the reduction of Cr(VI) to Cr(III) and subsequent preconcentration of Cr(III). Sample loading volume: 1.8 mL; Sample to reductant volume ratio: 4:1; Eluent volume: 25 μL; Cr(VI) concentration: 0.4 μg L^{-1}; Reduction time: 240 s (reprinted from ref. [III] with courtesy of The Royal Society of Chemistry).

Parameters concerning sample loading and elution of sorbed species were optimized. All the optimized experimental conditions for SI-LOV system and ETAAS parameters are summarized in Table 6-1.

Table 6-1. Experimental parameters for SI-LOV-ETAAS system for speciation analysis of Cr(III) and Cr(VI)

Pyrolysis temp./Ramp time+Holding time	1500 °C/10 s + 20 s
Atomization temp./time	2300 °C/4 s
Sorbent	Chelating Sepharose beads
Sample loading flow rate ($\mu L\ s^{-1}$)	75
Eluent	25μL of 0.1 mol L^{-1} HNO_3
Flow rate of elution ($\mu L\ s^{-1}$)	10
Total chromium measurement:	
Sample acidity	pH 3.8
Reductant for Cr(VI) to Cr(III) (mixed with sample at a 1:4 flow-rate ratio)	0.02 mol L^{-1} NH_2OH at pH 3.8
Reduction time	*ca.* 4.0 min
Cr(III) species measurement:	
Sample acidity	pH 3.8

6.3 Performance

In Table 6-2 is shown the analytical performance data for the system. As can be seen, the enrichment factor for Cr(III) is very high (62) – allowing for a very low detection limit – while the enrichment factor for Cr(VI) is somewhat lower (42). This is due to the fact that the reduction efficiency is "only" 68% (62 $\times$ 0.68 = 42), but since it is constant and reproducible it does not impair the measurement due to the exact and reproducible timing that is ensured.

A set of environmental waters as well as certified reference materials (*viz.,* SRM 1640-Natural Water and CRM-320 River Sediment) were analyzed for their Cr(III) and total chromium content to evaluate the applicability and accuracy of the proposed procedure (see Appendix 2-III). Tap, river and later water were spiked with two concentration levels of Cr(III) and Cr(VI) and the values found are listed in Table 6-3. As can be seen, satisfactory recoveries were obtained for the spiked waters. Statistical comparison of means between experimental results and the total chromium certified

values for the CRM and NIST materials revealed the non-existence of significant differences at a 95% confidence level.

Table 6-2. Analytical performance of the μSI-BI-LOV-ETAAS system using on-line reduction for Cr(III) and Cr(VI) speciation.

Parameter	**Cr(III)**	**Cr(VI)**
Regression equation	1.0792 [Cr(III), μg L^{-1}]	0.7380 [Cr(VI), μg L^{-1}] – 0.0008
Linear range (μg L^{-1})	0.02-0.28	0.035-0.4
Sample volume (mL)	1.8	1.8
Sample frequency (h^{-1})	12	8
Retention efficiency (%)	86	---
Reduction efficiency (%)	---	68
Enrichment factor	62	42
Detection limit (μg L^{-1}) (3σ)	0.010	0.020
Repeatability (%, 0.2 μg L^{-1}, n=7)	2.4	2.2
Reproducibility (%,0.2 μg L^{-1}, n=6)	4.7	4.5

Table 6-3. On-line determination of trace levels of hexavalent and trivalent chromium in natural waters by hyphenation of μSI-BI-LOV with ETAAS.

Sample	**Added (μg L^{-1})**		**Found (μg L^{-1})[b]**		**Recovery (%)**	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
Tap water[a]	---	---	0.027±0.002	< LOD	---	---
	0.03	0.05	0.059±0.001	0.048±0.003	104	96
	0.08	0.06	0.108±0.003	0.066±0.004	100	110
River	---	---	0.019±0.002	0.071±0.002	---	---
water[a]	0.04	0.08	0.054±0.002	0.16±0.01	92	106
	0.1	0.1	0.118±0.005	0.16±0.01	99	94
Lake	---	---	< LOD	< LOD	---	---
water	0.04	0.08	0.035±0.002	0.076±0.004	88	95
	0.1	0.1	0.101±0.005	0.104±0.002	100	104

[a] Dilution factor, 1:10

[b] The results are given as the mean of 3 replicates ± SD

7. SI-BI-LOV-ETAAS schemes for on-line dynamic extraction, separation and preconcentration, and determination of readily bioavailable Cr(VI) in solid substrates [V, VI]

7.1 Introduction

Pretreatment such as extraction/digestion is often a prerequisite for the chemical analysis of solid sample. In environmental studies, extraction methods intended for speciation/fractionation analysis - that is, the solid sample being exposed to an extracting reagent able to dissolve the targeted compounds or pre-defined physico-chemical phases of ecological interest [121] - have gained widespread acceptance because they can reveal relevant information regarding pollutant soil-phase associations as well as the elucidation of the mode of occurrence, magnitude of available reservoirs, and potential migration of elements in natural environments [122].

The third generation of flow injection analysis has proven to be an excellent approach for automation and miniaturization of liquid-phase assays and on-line handling of sorbent materials. Yet, it is not a simple task when it is extended to the application of manipulation of environmental solids, such as soils and sediments in a forward-backward fashion in the integrated microconduits of the LOV unit due to the consequence of the large size distribution of the solid particles, shape heterogeneity, and high solid to suspending solution density ratio. In addition, the limited capacity of the cavities in the LOV (< 20 mg soil) restricts the potential applicability of the third generation of flow injection to highly homogeneous solids in order to ensure sample representativeness.

To tackle the above-mentioned shortcomings, the versatility of the SI-LOV approach in terms of accommodation of peripheral modules at will according to the requirements of the analytical assay was exploited for on-line treatment and analysis of solid samples as contained in a specially designed micro-cartridge [123]. One interesting analytical application of this arrangement is the development and characterization of flow-through sequential extraction schemes for ascertaining the bioavailability, mobility and thus toxicity of trace elements in solid substrates by attacking defined chemical forms and metal soil-phase associations [123- 125].

However, miniaturised column-based dynamic extraction protocols have so far not received a broad appeal due to two main reasons: firstly, the minute capacity of in-line microcolumns for accommodating the solid sample and the build-up of backpressure for solid amounts ≥ 50-100 mg; secondly, the inherent difficulty of hyphenated systems for ascertaining the most ecotoxicological relevant forms of trace elements in environmental solids, i.e., the most readily leachable fractions, namely, water-soluble, exchangeable and mild acid-soluble.

The adoption of the dedicated dual-conical microcolumn developed in our group [123], not only ensures sample representativeness by admitting substrate amounts up to 300 mg without unduly pressure increase, but features fluidized-bed like conditions that result in appropriate mixing between sample and extractant [126]. Furthermore, the attachment of the sample container at a peripheral port of the multiposition valve facilitates the application of bi-directional flow with the subsequent mitigation of backpressure or clogging effects due to soil compaction, which are frequently encountered in continuous-flow or uni-directional flow injection fractionation manifolds [125].

Automated flow-systems with on-line detection have, however, been used solely for fractionation of elements in highly contaminated solid substrates, the raw extracts generated being in all instances delivered directly to the hyphenated analytical instrument [127- 130], mostly ICP-MS [127,128,130], without any prior sample treatment step. Hence, their applicability to highly salted matrices or to extracts containing ultra-trace metal contents is rather limited. Actually, these methods fail to

monitor the most ecotoxicological significant fractions of trace metals, i.e., the water-soluble or exchangeable pools, which determine the readily available, and, thus toxic, forms for biota uptake.

To circumvent the above drawbacks, an automated and rugged SI-LOV microcolumn fractionation system hyphenated to ETAAS detection and integrating on-line matrix separation and additional measurand preconcentration was therefore proposed, for the first time, for expeditious and accurate monitoring of the content of easily mobilisable hexavalent chromium in soil environments at the sub-low parts-per-million level utilizing distilled water and artificial acid rain as well.

7.2 Method development: Configuration, parameters and operational procedure description

The microflow arrangement, which is shown schematically in Fig. 7-1, integrates dynamic leaching of Cr(VI) using deionised water or artificial acid rain as single extractants; on-line pH adjustment of the extract to minimize undesired Cr(VI)/Cr(III) interconversions under the slight acidic medium of the aqueous extractants; isolation and preconcentration of the chromate leached from the matrix constituents and reagent medium onto beads freshly packed into the microconduits of the LOV assembly; air-segmented elution of the sorbed species which are detected by ETAAS; and finally withdrawal of the used beads for each step of the multiple extraction protocol to circumvent the progressive sorbent deterioration and the influence of irreversible interferences from the soil matrix [V]. In this configuration, the upright disposition of the soil microcolumn is intended to withhold the entire substrate in the lower conical cavity of the container for facilitating fluidized bed mixing conditions during the progressive outward pumping of the leaching reagent through the packed column as well as to strip quantitatively the extractant out of the moistened solid whenever the solution is pulled back toward the valve by the reverse

motion of the syringe pump.

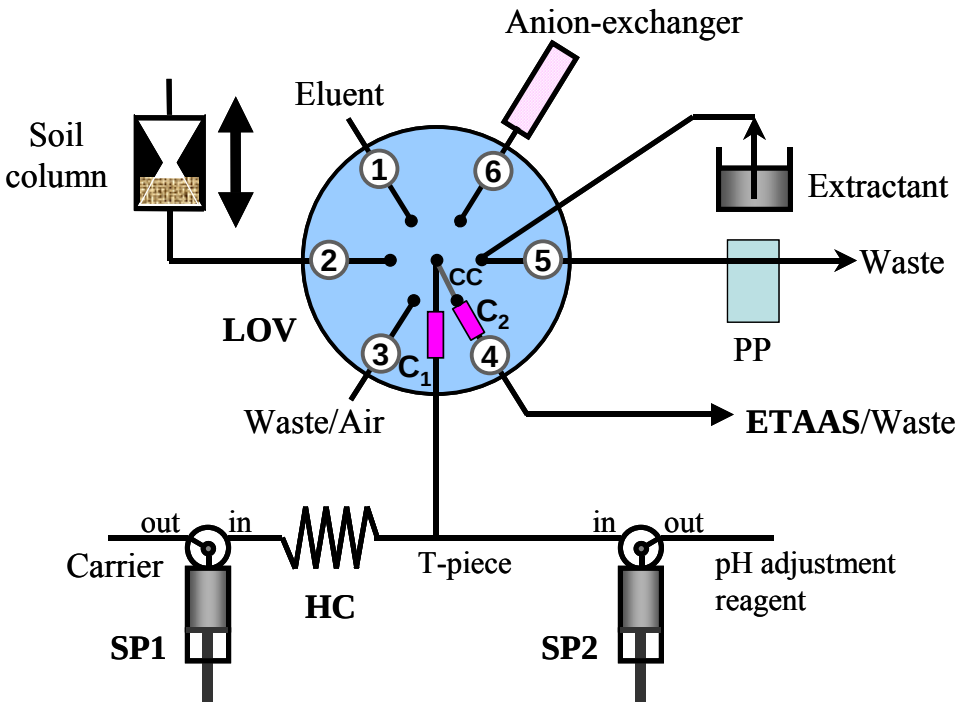


Fig. 7-1. Schematic diagram of the μSI-BI-LOV-ETAAS system for dynamic fractionation of Cr(VI) in environmental solids. Carrier, 0.01 mol L^{-1} Tris-HNO_3 buffer at pH 8.0; on-line pH adjustment reagent: 0.02 mol L^{-1} Tris-HNO_3 buffer at pH 8.0; Eluent, 0.5 mol L^{-1} NH_4NO_3 /NH_4OH at pH 8.0; Beads, Q Sepharose; SP1/SP2, Syringe pumps 1 and 2; C_1 and C_2, LOV microcolumn positions; HC, Holding coil; CC, Central communication conduit; PP, Peristaltic pump, ETAAS, electrothermal atomic absorption spectrometer (reprinted from ref. [V] with courtesy of The Royal Society of Chemistry).

The optimization of the various operational and chemical parameters, bead material, sample acidity, loading flow rate, eluent type and stripping conditions and tolerance to potential interfering species was conducted using aqueous solutions (attached to port 2) in lieu of soil extracts.

In order to isolate Cr(VI) from Cr(III) prior to its presentation to the detector, anion-exchangers were exploited as a sorptive medium for Cr(VI) taking into account its anionic nature in most natural environments. Among the various sorbents exploited in a permanent fashion for Cr(VI) enrichment, namely Dowex 1-X8 [131-133] and Sepharose/Sephadex-type [134] exchangers, the latter ones are preferable for handling in the LOV unit as renewable surfaces due to their hydrophilic nature,

perfectly spherical shape and narrow size distribution. Although QAE-Sephadex showed appropriate adsorption performance for Cr(VI) species, its utilization as microcolumn material in LOV cavities is limited by the volume changes of the resin-bed as a consequence of sorbent shrinking/swelling upon application of solutions of different composition and/or ionic strength. Thus, Q-Sepharose, which is physically more resistant than Sephadex exchangers and can be directly manipulated in the flow network with no need for any ancillary treatment, was selected for Cr(VI) enrichment in the LOV.

For Cr(VI), the anionic forms are the prevalent species at pH> 2.8. Yet, the tolerance to potentially interfering monovalent anions in real-life samples was improved at pH ≥8.0 as a consequence of the stronger affinity of the resin for the predominantly divalent chromate oxoanion. Moreover, slightly alkaline media are commonly recommended for stabilization of Cr(VI) solutions [132,135]. Therefore, buffering of the standards was accomplished by addition of Tris-HNO_3 buffer (pH 8.0). Significant chromate breakthrough (> 20%) was detected for buffer concentrations above 0.05 mol L^{-1} Tris as a result of the pre-elution effect occasioned by the surplus of nitrate. Finally a 0.01 mol L^{-1} Tris-HNO_3 buffer solution was selected as a compromise between Tris buffer capacity and retention efficiency for Cr(VI).

Different kinds of eluents such as mineral acid (HNO_3) and electrolyte buffer (NH_4NO_3-NH_4OH) for stripping the retained species from packed-bead ion-exchange column were evaluated. Improvement of analytical performance was observed for the latter one at alkaline condition (i.e., pH 8.0). Alkaline media minimizes the possible oxidation of the organic groups of the matrix beads.

Parameters concerning sample loading flow rate, eluent volume and elution flow rate were optimized. The optimized experimental conditions for SI-LOV system and ETAAS parameters are summarized in Table 7-1.

The influence of the prevailing water soluble anionic species in soil extracts, such as Cl^-, HCO_3^-, NO_3^-, SO_4^{2-} and the most ubiquitous cationic species, such as Ca^{2+} and Mg^{2+}, which might lead to non-spectroscopic interferences during analysis, was evaluated. Experimental results showed that the ion-exchanger materials can endure

monovalent anions such as Cl^-, HCO_3^- and NO_3^- at the ratio of interferent/Cr(VI) ranging from 5×10^6 to 1×10^7. As for the potential interfering effect of Ca^{2+} and Mg^{2+} on the detection instrument, it should be born in mind that cationic species are not sorbed on the anionic-exchanger so that concentrations as high as 200 mg L^{-1} are admissible for accurate dynamic fractionation of Cr(VI).

Table 7-1. Experimental parameters for SI-LOV fractionation system for the determination of readily bioavailable Cr(VI) in solid substrates by ETAAS.

Pyrolysis temp./Ramp time+Holding time	1500 °C/25 s + 20 s
Atomization temp./time	2300 °C/4 s
On-line dynamic fractionation:	
Extractant	Deionised water, simulated acid rain
Extraction flow rate (μL s^{-1})	Pump forward: 50; Pull inward: 7
Separation and preconcentration of Cr(VI) by μSI-BI-LOV:	
Sorbent	Q-Sepharose, strong anionic exchanger
pH of extract after pH adjustment	pH 8.0
Loading flow rate (extract + pH adjustment solution) (μL s^{-1})	100
Eluent	40 μL of 0.5 mol L^{-1} NH_4NO_3-NH_4OH (pH 8)
Flow rate of elution (μL s^{-1})	10 + stopped-flow (7 s)

7.3 Performance

In Fig. 7-2 is depicted an example of the multiple-step dynamic extraction profile for assessment of the readily bioavailable content of Cr(VI) in soils as obtained by attacking a moderately polluted soil material (SRM 2709) and various spiked batches with mild extractants. For this particular case, the progressive acidification of the extraction media did not increase the leachability of soluble Cr(VI) from the sample, which is attributed to the efficiency of distilled water for quantitative removal of soluble (surface bound) chromate in an on-line dynamic mode, and the ability of the soil material to raise the pH of the applied extractant, thereby precluding the additional release of sparingly-soluble forms of Cr(VI).

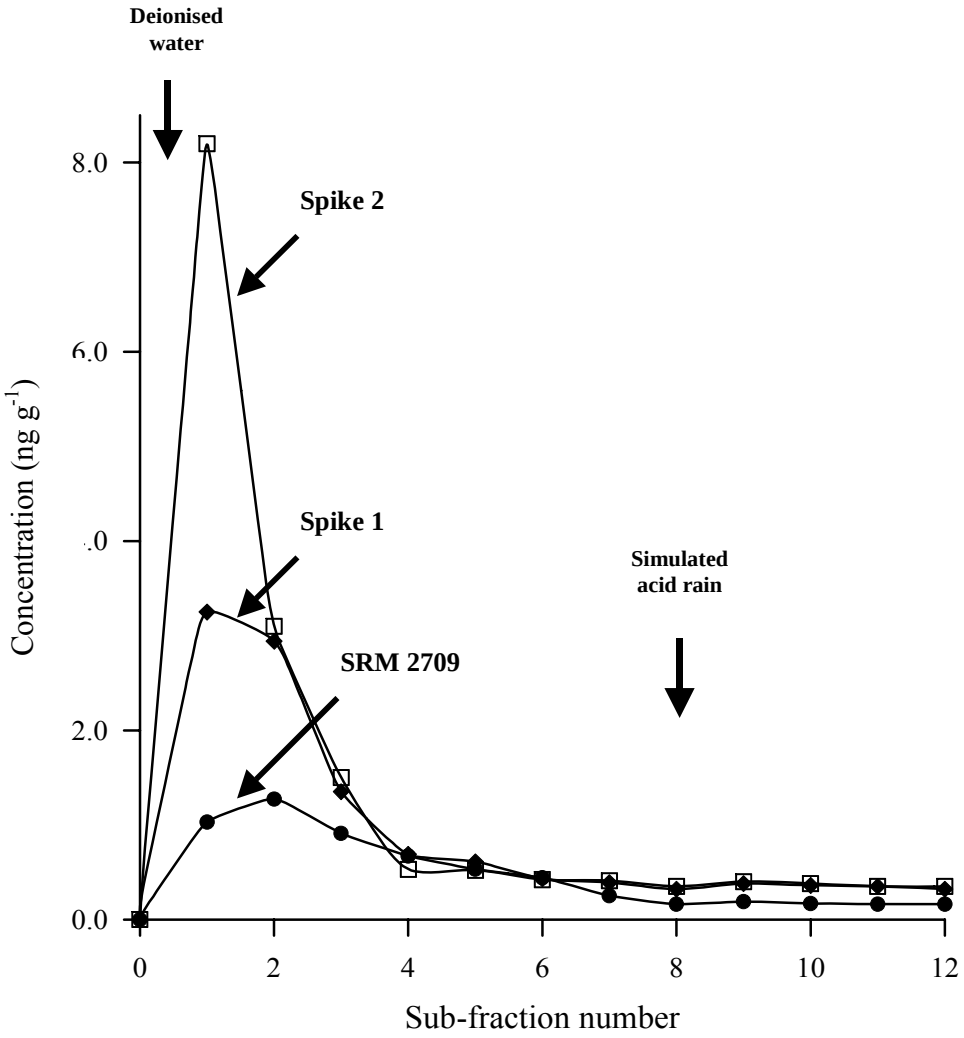


Fig. 7-2. Extraction profiles of readily bioavailable Cr(VI) in SRM 2709 and spiked samples as obtained from the SI-LOV microcolumn system using mild extractants. Soil amount, 100 mg; sub-fraction volume, 500 μL; Spike 1, 5.0 ng g^{-1}; Spike 2, 8.0 ng g^{-1} (reprinted from ref. [V] with courtesy of The Royal Society of Chemistry).

The reliability of the proposed method was evaluated via fortification of a moderately polluted agricultural soil material (SRM 2709) with water-soluble Cr(VI) salts at different concentration levels. Experimental results are compiled in Table 7-2.

The potential extension of the SI-LOV analyzer for speciation/fractionation of Cr(VI) and sample clean-up in highly polluted samples has also been assessed by using the miniaturized unit as a front end to FAAS rather than ETAAS. Despite the continuous operation nature of the detection instrument and the discontinuous flow inherent to SI, hyphenation between both set-ups can be easily realized by interfacing a rotary injection valve for continuous injection of the extracts into the FAAS nebulizer

stream [126]. As a result of the flexibility of the SI-LOV-AAS coupling, environmental solids with variable amounts of available Cr(VI) ranging from the sub-μg kg^{-1} to the mg kg^{-1} level, i.e., above the maximum permissible concentrations for agricultural use, may be automatically treated and further analyzed in the fully enclosed flow assembly.

Table 7-2. Water extractable concentrations of hexavalent chromium for SRM 2709 and different soil spikes at variable concentration levels using the μSI-BI-LOV microcolumn set-up.

Soil sample	Amount/mg	Added/ng g^{-1}	Found/ng g^{-1}	Recovery (%)
SRM2709	100	---	4.9±0.3	---
Spike 1	100	5.0	9.5±0.5	96±5
Spike 2	100	8.0	13.9±0.6	108±4
Spike 3	20	40	44±3	98±6
Spike 4	20	55	64±2	107±3

Results are expressed as the mean of 3 extraction replicates ± SD

8. Multisyringe flow injection LOV system coupled to atomic fluorescence spectrometry for online preconcentration and determination of hydride-forming elements in environmental waters [VIII]

8.1 Introduction

In comparison with AAS, atomic fluorescence spectrometry (AFS) features improved performance as regards to the minimization of light scattering effects and background matrix interferences [136] and yielding, commonly in hyphenation with hydride generation (HG) systems, of better detection limits for hydride forming species [137].

However, HG-AFS, when applied to direct analysis of some complex environmental samples, has been proven rather cumbersome due to the ultra-low concentration levels of metalloids in the samples and to the interference from concomitant transition metal ions [138]. As an example, the presence of certain metal ions, particularly Cu, Ni and Co, can react with the reducing reagent, tetrahydroborate, and be reduced to colloidal free metal particles or metal borides. These constituents are superb catalysts for degrading the hydrides even before they reach the gas-liquid separator, thereby posing as potentially severe interferences. Therefore, ancillary sample pretreatment procedures are employed to remove interfering sample constituents and to preconcentrate target measurand.

The marriage between the sample pretreatment scheme of SI-LOV and HG-AFS for assays of trace level metalloids has not been reported to date due to the ostensible hindrance of the discontinuously operating μSI systems for implementation of post-LOV derivatization reactions. Therefore, the third generation of flow injection has been so far mostly applied to on-column spectroscopy [139-141], where the bead column is trapped within the in-valve flow cell and continuously monitored by

UV-Vis spectroscopy or fluorometry, or to atomic spectrometry measurements based on the injection of the measurand containing eluate directly into the detector without further processing [63, III,IV].

This chapter is devoted to explore the performance and potential applicability of the hyphenation of LOV with AFS detection through the recently introduced multisyringe flow injection (MSFI) approach [142- 144] for automatic on-line sample treatment by the renewable solid-phase extraction approach and subsequent derivatization of the eluate for monitoring of trace levels of hydride forming species. MSFI combines the advantages of multichannel operation with the use of a suite of syringes of variable volume, which ensures a constant, pulseless flow as well as the accurate metering of microvolumes of solutions via multicommutation protocols.

The determination of total inorganic arsenic has been selected as a target measurand for the demonstration of the analytical potentials of such hyphenation. Arsenic is ubiquitous in the environment due to natural sources and widespread anthropogenic use as a pesticide and herbicide, growth promoter for swines, food additive to combat deceases in poultry as well as preservative for wooden structures. Inorganic arsenic, including As(III) and As(V), dominates in freshwaters with concentrations of various orders of magnitude higher than those of organic forms [145]. The toxicity of arsenic strongly depends on its chemical forms, inorganic arsenic species being more toxic than the organic ones [146]. According to the accumulation of evidence for the chronic toxic effects of inorganic arsenic [145,146], the regulatory limits from the World Health Organization (WHO), the United States Environmental Protection Agency (US-EPA) and the European Community (EC) in drinking water are set at 10 μg/L [147].

The increased awareness of the presence of toxic inorganic arsenic in the environment, and the need for routinely determination of its low concentrations in natural waters have, thus, made the development of ultra-highly sensitive, automated, and affordable methods imperative.

8.2 Method development: Configuration, parameters and operational procedure description

In neutral or slightly alkaline milieu, As(V) dominantly exists as oxoanionic forms while As(III) exists as neutral or monovalent oxoanionic form. Hence, a strong anion exchanger is a potential sorptive material for the on-line preconcentration of inorganic arsenic in water. Consequently, Q-Sepharose resin, which has ideal physical properties and has been proven highly efficient for collection of ionic species in LOV [V], was adopted.

Experimental results showed that As(III) was not well retained on the beads, possibly due to the small dimension of sorptive column and single charge of oxoanion. On the other hand, As(V) exists in solution predominantly as multicharged species at pH ≥ 7.0, which might facilitate the improved uptake by ion-exchange processes. Therefore, an oxidation agent (potassium permanganate) was added to the sample for the conversion of As(III) into As(V) followed by pH adjustment to facilitate the uptake of arsenic by the ion-exchange processes.

After isolation of inorganic arsenic from the matrix constituents by preconcentration on the sorptive material, As(V) is subjected to two operations prior to final quantification by HG-AFS, that is, firstly, the collected As(V) is stripped out from the LOV microcolumn, and secondly, the As(V) is derivatized into a gaseous hydride of arsenic upon reaction with a reducing agent. However, the reduction rates [148, 149] in direct evolvement of arsine from arsenate is low and hence a pre-reduction step to reduce As(V) to As(III) prior to arsine generation is often indispensable [150,151].

In this context, a combined reagent (10% KI in 6 M HCl) was proposed as a pre-reductive eluent for both the removal of As(V) from the LOV packing and concomitant on-column derivatization at room temperature without any incubation of the reactant zone. The acidic medium in the eluent not only ensures protonation of the target anionic species, making the neutral substances inaccessible for the quaternary

ammonium moieties of the sorbent, but also facilitates the conversion of As(V) into As(III) and hydride generation [151], while the potassium iodide, on one hand, increases the eluting strength of the reagent, and, on the other hand, fosters the pre-reduction of the collected As(V).

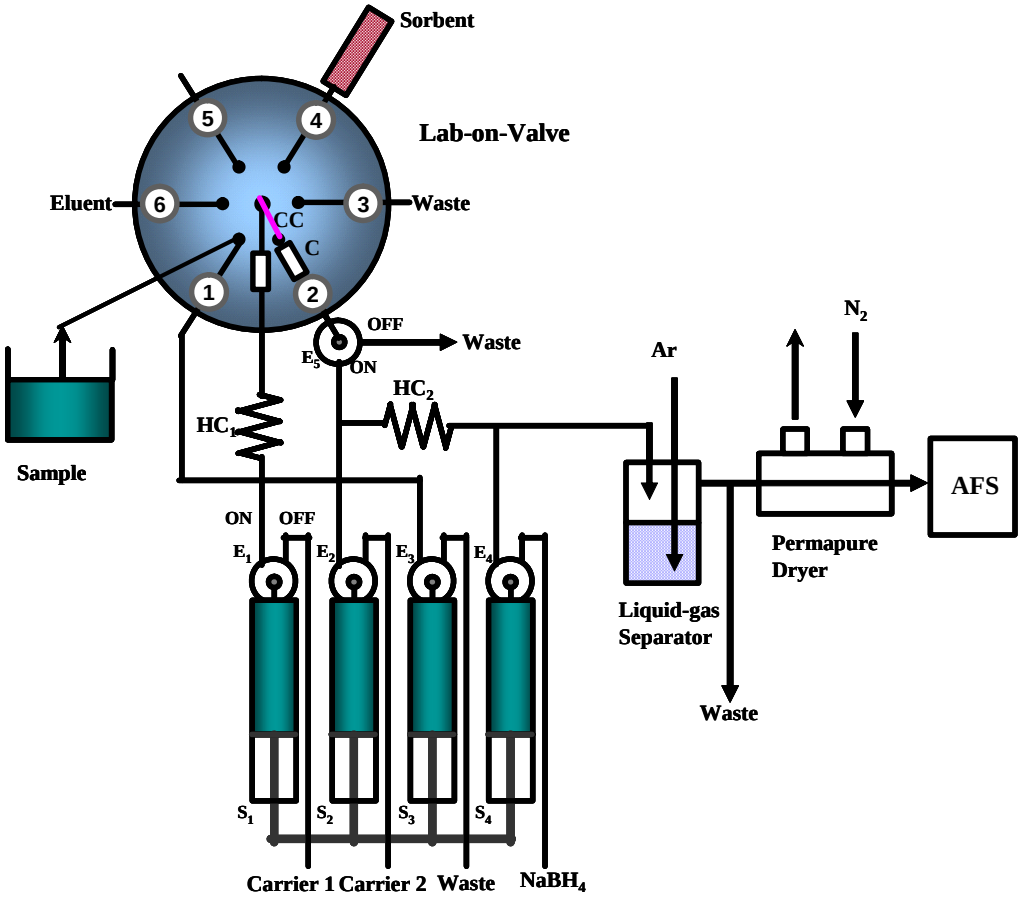


Fig. 8-1. Schematic illustration of MSFI-LOV manifold hyphenated to HG-AFS for the BI preconcentration and determination of trace level concentrations of total inorganic arsenic. Carrier: 0.01 mol L^{-1} ammonium chloride/ammonia buffer at pH 10 + $8x10^{-5}$ mol L^{-1} citrate; $NaBH_4$: 0.3% (w/v); Eluent: 10% KI + 6 mol L^{-1} HCl + 0.2% ascorbic acid; S_1-S_4: Syringes; V_1-V_5: Solenoid valvea; T_1 and T_2: Three-way-connectors; HC_1 and HC_2: Holding coils; C_1 and C_2: LOV micro-column positions; CC: Central communication conduit; AFS: atomic fluorescence spectrometer (reprinted from ref. [VIII] with courtesy of The American Chemical Society).

The MSFI-LOV assembly hyphenated to HG-AFS is schematically illustrated in Fig. 8-1. In operation [VIII], following the loading of beads in the C_2 position, sample is pumped to C_2 for effecting the preconcentration of As(V) in the micro column and discarding the used sample solution to waste via solenoid valve V5. Afterwards, an eluent plug is dispensed forward to C_2 for quantitative stripping of the collected As(V)

with concomitant on-column reduction to As(III). Then, the eluate plug, driven by water from S2, is merged with sodium tetrahydroborate solution from S4 and delivered to the gas-liquid separator for completion of the reaction and isolation of the evolved arsine, which is guided into the flame of the AFS via a stream of inert carrier gas for detection.

Optimized parameters concerning sorbent, sample loading, pre-reductive elution and HG are list in Table 8-1.

Table 8-1. Optimal operational parameters of the MSFI-LOV-HG-AFS system for the determination of total inorganic arsenic

Parameters	**Value**
Sample volume (mL)	3.0
Oxidation reagent of sample	2×10^{-6} mol L^{-1} $KMnO_4$
Sample pH	pH 10
Loading rate (mL min^{-1})	3.0
Eluent/pre-reductive reagent concentration	10%KI+ 6 M HCl +0.2% ascorbic acid
Eluent/pre-reductive reagent consumption (mL)	0.3
Elution flow rate (mL min^{-1})	1.0
$NaBH_4$ concentration (%)	0.3
$NaBH_4$ consumption (mL)	0.75
Sorbent amount (mg)	5.0±0.5

8.3 Performance

The analytical figures of merit of the MSFI-LOV hyphenated system obtained under the optimized chemical and physical conditions are compiled in Table 8-2.

Table 8-2. Analytical performance of the MSFI-LOV-HG-AFS system for the determination of total inorganic arsenic

Regression equation	IF* = 359.27 [As, ng mL^{-1}] + 24.93
Linear range (ng mL^{-1})	0.05-0.3 (for an 800-fold AFS gain)
Detection limit (ng mL^{-1}, n=9, $3\sigma_{blank}$)	0.02
R.S.D. (%, 0.1 ng mL^{-1}, n=7)	5.7
Enrichment factor	8.8
Sample frequency (h^{-1})	9.0

*: relative fluorescence intensity

As compared with earlier on-line HG-AFS methods based on the exploitation of the various generations of flow injection analysis [151- 153], the present LOV hyphenated set-up yields a 5-fold improved analytical sensitivity and the enhancement of the detection limit by more than one order of magnitude. Furthermore, the MSFI-LOV approach should be viewed as an environmentally friendly analytical method as a result of the minimum consumption of aggressive chemicals as regards to batch but flow injection analysis systems as well. Actually, the amount of $NaBH_4$ and HCl delivered per assay is 24 and 24-fold, respectively, reduced as compared with the first generation of flow analysis using commercial system by PS Analytical Ltd [152].

Concomitant transition metals in the sample matrix can cause the most severe side reactions in the evolving of gaseous hydrides of metalloids because metal ions, particularly Ni, Cu and Co, are known to react with tetrahydroborate, and become reduced to colloidal free metals or metal borides [154] which have proven to be superb catalysts for degrading of the hydrides before reaching the detector, while the colloidal particles also are efficient media for adsorption of the volatile compounds [155]. Yet, an elegant approach, here selected, for circumventing the above interfering effects, is to collect the target oxoanions onto anion-exchanger resins, thus facilitating the isolation of the hydride forming measurands from positively charged species, such as transition metal ions. The maximum tolerated concentration of anions commonly encountered in environmental waters was ascertained. The tolerated interferent/measurand ratios of nitrate, chloride, hydrogen carbonate/carbonate and sulfate were 5×10^6, 2.5×10^6, 5×10^5 and 5×10^5, respectively. As compared with a recently reported flow injection-HGAAS set-up for the determination of trace level concentrations of inorganic arsenic following sorptive ion-exchange preconcentration [156], the LOV-MSFI hyphenated system yields a 5 to 10-fold improved tolerance to the overall anionic species assayed. The tolerance to hydride forming elements was also evaluated. In fact, ratios of Se(IV) to As $\leq$ 5000 and Sb(V) to As $\leq$ 500 were tolerated at the 10% interference level. In comparison with previous flowing stream methods for direct determination of arsenic in the liquid phase via HG-AFS [151], the proposed method features improved tolerance for Sb and Se by 20 and 250-folds,

respectively.

Environmental water samples, including tap and underground water, as well as certified reference materials of relative matrix complexity (viz., ERM-CA010-Hard drinking water and TMDA-54.3-Lake water) were utilized to ascertain the reliability and accuracy of the proposed MSFI-LOV preconcentration method. No significant differences were found between the experimental results and the certified values at a significance level of 0.05. The recoveries obtained for the environmental waters spiked with two levels of arsenate below the regulatory limits endorsed by the WHO for arsenic in drinking water (i.e., 10 ng mL^{-1}) are listed in Table 8-3. Satisfying recoveries were achieved for the spiked environmental waters.

Table 8-3. Determination of trace level concentrations of inorganic arsenic in environmental waters exploiting MSFI-BI-LOV in hyphenation with HG-AFS

Sample	**Added (ng mL^{-1})**	**Found (ng mL^{-1})**	**Recovery (%)**
Tap water (Valldemossa, Spain)	0	< LOD	---
	1.0	0.93±0.05	93
	1.5	1.65±0.05	110
Underground water (Palma, Spain)	0	0.23±0.03	---
	1.0	1.29±0.03	105
	1.5	1.8±0.1	104

The results are expressed as the mean of three replicates ± SD

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Uncertainty budget for the project

"On-line separation and preconcentration of chromium (VI) in water by diphenylcarbazide loaded C18-PS/DVB beads in Sequential Injection Lab-on-Valve followed by measurement with electrothermal atomic absorption spectrometry (ETAAS)"

Contents

1 Introduction

In the research project a new concept is presented for selective and sensitive determination of trace metals via electrothermal atomic absorption spectrometry (ETAAS) based on the principle of bead injection (BI) with renewable reversed-phase surfaces in a Sequential Injection-Lab-on-Valve (SI-LOV) mode. The methodology involves the use of poly(styrene-divinylbenzene) beads containing pendant octadecyl moieties (C_{18}-PS/DVB), which are pre-impregnated with a selective organic metal chelating agent prior to the automatic manipulation of the beads in the microbore conduits of the LOV unit. The potential of the SI-BI-LOV scheme is demonstrated by taking Cr(VI) as an example, using a 1,5-diphenylcarbazide (DPC) loaded bead column as the active microzone. The proposed procedure was successfully applied to the determination of trace levels of Cr(VI) in environmental water samples containing high levels of dissolved salts.

This budget describes the detailed procedure for the evaluation of uncertainty of the measurand of the project.

2 Specification of measurand

2.1 Experimental

2.1.1 Reagents and solution preparation

All chemicals are of analytical-reagent grade and doubly de-ionised water (18.2 MΩ cm) obtained from a Millipore system (Milllipore Synthesis A10, France) is used throughout for solution preparation. All glassware is rinsed prior to use with a 25 % (v/v) concentrated nitric acid solution utilising a washing machine (Miehle, Model G 7735 MCU, Germany), and afterwards cleansed with Milli-Q water.

2.1.1.1 Preparation of calibrants

5 calibrants with concentrations ranging from 0 to 0.672 μg/L of Cr(VI) are daily freshly prepared by appropriate dilution of a 1000 mg L^{-1} of chromate (CrO_4^{2-}) stock solution (Merck, re Appendix 2) in water.

Firstly, 4.483 mg L^{-1} of Cr(VI) is obtained by diluting 1 mL of 1000 mg L^{-1} chromate stock solution to 100 mL in a 100 mL flask.

Secondly, 44.83 μg/L of Cr(VI) is obtained by diluted 1 mL of 4.483 mg L^{-1} Cr(VI) solution to 100 mL in a 100 mL flask.

Aspirating0, 0.30, 0.50, 1.00, 1.50 mL of above mentioned 44.83 μg/L Cr(VI) solution and diluting to 100 mL with water respectively, so four calibrants with concentrations of 0, 0.134, 0.224, 0.448 and 0.672 μg/L are obtained.

2.1.1.2 Sorbent preparation

Prior to reagent immobilisation, a 1:20 C_{18}-PS/DVB (w/v) bead suspension is prepared in methanol and filtered through a glass filter (15-40 μm) to remove particles smaller than 40 μm that cannot be quantitatively entrapped within the LOV microcolumn cavities. The bead material retained on the filter is cleansed with two 5 mL portions of methanol, and finally vacuum-dried for 5 min. To prepare the active surfaces for Cr(VI), 1.0 mL of a 3.6

% (m/v) DPC solution in methanol is added to 0.2 g of pre-cleansed beads. A final concentration of 5% (v/v) methanol is ensured by adding 250 μL of methanol before bringing the suspension volume to 25 mL with water. The resulting suspension is preserved from light and subjected to continuous stirring for 30 min. The adsorption process of the reagent onto the bead surfaces can be followed by the naked eye because of the developing characteristic dark pink colour of the DPC-modified sorbent. The sorptive material is aspirated into a 1-mL plastic syringe, which then is mounted vertically on port 6 of the integrated microsystem. After settlement on the bottom of the reservoir, the DPC-loaded beads are readily handled via SP1 into the various positions of the LOV unit. The impregnated beads can be used for less than two days, provided that they are stored refrigerated at 4ºC whenever not used.

2.1.1.3 Other solutions preparation

The carrier consisted of 0.5 mol L^{-1} HNO_3, while on-line pH adjustment is achieved by pumping a 5 mol L^{-1} HNO_3 solution by means of the external syringe pump SP2. A 90% (v/v) methanol/water solution is employed as eluent.

2.1.2 Operational procedure

The pre-filtered (by 0.45 μm pore size membrane) environmental water sample (*ca.* 1 liter) is stored in a container and avoided exposure to air. Sample and calibrants are stirred by magnetic bar for 1 minute before introduction to the SI-BI-LOV system. The same SI-BI-LOV method is applied to all calibrants and the sample. All the calibrants and sample are measured by duplicate sampling. The measurement of samples is conducted after the measurement of freshly prepared calibrants to minimize the influence of instrumentation condition from different days. For a batch of samples with population of more than 10, a calibrant is measured to check the stability of system every 10 measurements of samples.

The schematic drawing of the system is presented in the paper (See ref. IV). The SI-BI-LOV method for handling of the reagent loaded C_{18}-PS/DVB beads involves five different steps, namely, system preconditioning, Cr(VI) sorption onto the modified hydrophobic entities through derivatization reaction, removal of matrix constituents and not retained chromium (re Equation (1)), elution of retained chromium (re Equation (1)), and finally bead disposal. The operational details of a complete measuring cycle (see ref. IV) including flow rates and volumes handled, selected ports of the SV, and positions of SP valves are summarized as follows:

System preconditioning (step 1). Initially, the HC is washed with 2500 μL of carrier (0.5 mol L^{-1} HNO_3) and the 8-cm line connecting SP2 with the T-connector is filled with the pH-adjustment acid solution. After these preliminary operations, SP1 is set to aspirate consecutively carrier solution from the external reservoir and methanol solution from port 1. Thereafter, the solvent segment plus 300 μL of carrier are, via the communication channel, directed to port 4 for rinsing column positions C1 and C2 and the connecting line to the ETAAS detector. Thus, a volume of 750 μL carrier volume is left in SP1 for subsequent use, i.e., sample clean-up and bead removal (re below).

Bead aspiration and sample loading (step 2). Firstly, 2 mL of sample is drawn to HC through port 5 from the sample container, at the same time pH adjustment solution is filled to SP2 from pH adjustment reagent container. A small air segment is then aspirated

into HC to place the head of the sample plug just exactly behind the T-confluence for appropriate subsequent on-line merging of sample with acid solution. A metered portion of the DPC-coated beads is next aspirated slowly into microcolumn C1. The communication channel is then connected to the peripheral port 4, and SP1 and SP2 are activated simultaneously. As a result, Cr(VI) reacts with the immobilized DPC in an acid environment. During the solid-phase derivatization reaction and preconcentration of Cr(VI), the beads in microcolumn C1 are concomitantly transferred to the C2 position.

Sample clean-up (step 3). For the removal of weakly or non-retained matrix constituents from the hydrophobic material, an amount of 500 μL of the previously stored carrier solution is propelled to port 4 to cleanse both the micro-column and the ETAAS line after sample loading.

Elution (step 4-6). To fulfil the accommodation volume requirements of the graphite tube and to preserve the identity of the eluate zone, the air-segmented elution approach was selected for transportation of the eluate into the ETAAS instrument. To this end, a gentle stream of aspirated air is initially used to replace the carrier solution in the ETAAS line. The ETAAS temperature program, which was adapted from the manufacturer's recommendations, is activated at this instant. Subsequently, SP1 is set to aspirate 30 μL of methanol solution into HC, which then is dispensed forward to slowly strip out the chromium chelate ($[Cr(HL)_2]^+$) (re Equation (1))on the beads from microcolumn C2. The metal chelate enriched zone is finally propelled by a second air segment into the graphite furnace via the autosampler tip.

Bead discarding (step 7). Once the autosampler tip has been moved out of the furnace, the un-coated C_{18}-PS/DVB beads are discarded by transferring them back to microcolumn C1 as a sorbent-methanol suspension, and afterwards delivered to waste through port 3.

The ETAAS program is synchronized with the SI-BI-LOV protocol, whereby the next sample starts to be processed automatically in the flow network while the former one is being pyrolysed and atomized in the furnace.

2.2 Chemistry concerned

Though the reaction between Cr(VI) and DPC develops fast merely at high concentrations of mineral acids (ca. 1.0 mol L^{-1} H^+)sample acidification is reported to bias the analytical results in natural waters due to the progressive reduction of the hexavalent chromium to Cr(III) by dissolved organic matter. Hence, in the proposed method automated pH adjustment of the sample plug to obtain a final acidity of 1.0 mol L^{-1} H^+ in the sample is attained immediately prior to its reaching the DPC-coated beads, as loaded in the LOV unit.

The balance shift of the equilibriums between various forms of Cr(VI) ($HCrO_4^{1-}$, CrO_4^{2-} and $Cr_2O_7^{2-}$) is pH dependent.

The derivatization reaction for Cr(VI) (chromate) with DPC in a strongly acid medium is reported to constitute a two-step process involving the oxidation of DPC (H_4L) to diphenylcarbazone (H_2L) by Cr(VI) via formation of a metastable metal-reagent intermediate with a special sterical conformation, and finally the chelation of the oxidized reagent with the generated Cr(III) species according to the following reaction (re Sandell, E.B.; Onishi, H. *Photometric Determination of Traces of Metals*, 4th ed.; John Wiley & Sons: New York, 1978; Vol. 3. P390):

$$2CrO_4^{2-} + 3H_4L + 8H^+ \rightarrow 2[Cr(VI)\text{-}H_4L] + H_4L$$
(beads) (beads)

$$[Cr(HL)_2]^+ + Cr^{3+} + H_2L + 8H_2O \quad (1)$$
(beads)

Therefore maximum 50% of Cr(VI) in the sample can be retained on the surface of the beads through oxidation state Cr(III) and subsequent chelate formed with diphenylcarbazone. The Cr(VI) which does not participated in the reaction, the Cr(III) product in the above mentioned reaction as well as the matrix go to the waste.

A washing step employing carrier solution (0.5 mol L^{-1} HNO_3) is introduced after sample loading to wash away everything which is not retained in the complex (re Equation (1)), and matrix elements which possible interfere the determination of chromium in ETAAS.

After sample loading, the beads containing $[Cr(HL)_2]^+$ is eluted by 30 μL of methanol solution. All of the chelate ($[Cr(HL)_2]^+$) is stripped from the beads and the eluate is transported into graphite tube of ETAAS for detection.

At the step of atomization in the ETAAS temperature program, the chromium in the form of $[Cr(HL)_2]^+$ in the graphite tube is atomized and detected.

The stages of the procedure are shown in Fig. 1:

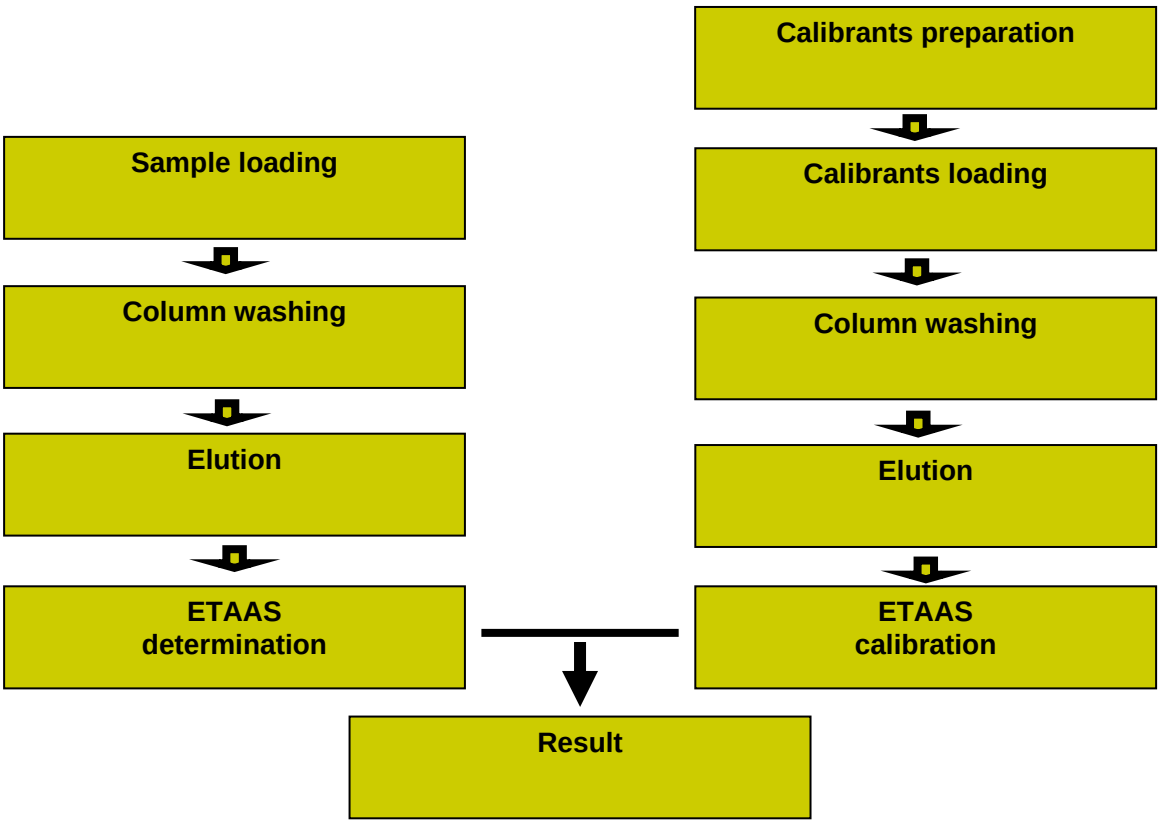


Fig. 1 Operating procedure

2.3 Measurand

2.3.1 Definition of the measurand

Total mass of soluble hexavalent chromium ($HCrO_4^{1-}$, CrO_4^{2-} and $Cr_2O_7^{2-}$) in ca. 2 mL (accurately measured volume) of sample at the time of being taken from a container with ca. 1 liter of pre-filtered (by 0.45 μm pore size membrane) environmental water sample, μg, m_0.

2.3.2 Expression for measurand:

$$m_0 = c_0 \times v_0 \times f_h \times f_f \times f_r \times f_e \times f_a \times 10^{-3} + \delta_i + \delta_w \qquad (2)$$

Where c_0 is calculated from the regression equation: $x_0 = a + bc_0$.
All the parameters used are compiled in Appendix 1.

3 Identification of uncertainty sources

Ishikawa diagram (Fig. 2):

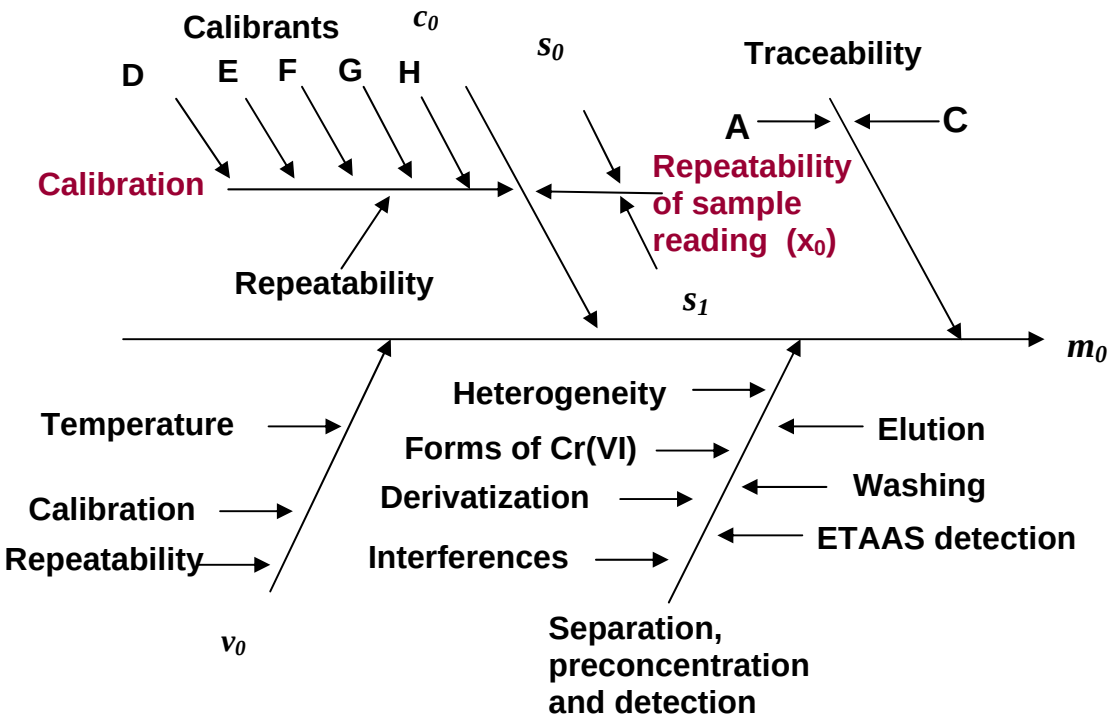


Fig. 2 Cause and effect diagram

Uncertainty sources:
1). Uncertainty from sample concentration c_0: $u(c_0)$, it includes three components:
(1) Repeatability of the reading of measured sample: $u(x_0)$
(2) Uncertainty from calibrants D, E, F and G: *u(c(D), u(c(E), u(c(F) and u(c(G)* , that is, from alteration of volumes of flasks and pipetman due to the influence of temperature, calibration and repeatability
(3) Repeatability of the reading of measured calibrants: *u(x)*

2). Uncertainty from volume of loaded sample v_o: $u(v_0)$
Variation of the volume in the aspiration of sample solution driven by a high precision syringe pump, it has three influences:
(1) Calibration
(2) Repeatability
(3) Temperature in the lab

3). Uncertainty in separation, preconcentration and detection procedure, it includes:
(1) Variation of heterogeneity of sample: $u(f_h)$
(2) Influence of the forms of Cr(VI) in the sample right before loading: $u(f_f)$
(3) Influence of the derivatization of Cr(VI): $u(f_r)$
(4) Variation of interference from the interfering ions in the sample matrix: $u(\delta_i)$
(5) Variation of washing step after sample loading: $u(\delta_w)$
(6) Variation of elution: $u(f_e)$
(7) Influence of running ETAAS temperature program: $u(f_a)$

4). Traceability
(1) Uncertainty from concentration of the original stock solution A: *u(C(A))*
(2) Uncertainty from concentration of the intermediate stock solution C: *u(C(C))*

4 Quantification of uncertainty components

4.1 Uncertainty from c_o

4.1.1

Repeatability of the reading of measured sample, $u(x_0)$
The detailed results of repeatability of level 0.448 μg/L of Cr(VI) and blank are listed in Table 1 and Table 2:

Table 1 Absorbance of 0.448 μg/L of Cr(VI**)**

Abs1	Abs2	Abs3	Abs4	Abs5	Abs6	Mean of Abs	Repeatability
0,1611	0,1605	0,1482	0,1582	0,1483	0,1579	0,1557	0,005904

Table 2 Absorbance of blank

Abs1	Abs2	Abs3	Abs4	Abs5	Abs6	Abs7	Mean of Abs	Repeatability
0,0529	0,0411	0,0470	0,0521	0,0438	0,0464	0,0462	0,0471	0,00422

The relationship of repeatability and the levels of sample:

Using the mean of absorbance (x) and repeatability ($u(x)$) data in Table 2 and 3 to estimate s_0 and s_1 in the following formula:

$$u^2(x) = s_0^2 + (x * s_1)^2 \qquad (3)$$

So
$s_0 = 0.0040$
$s_1 = 0.0278$

For the sample, the means of the sample reading x_0 is 0.1001 (0.1029, 0.0973), $u(x_0)$ is calculated by expression:

$$u^2(x_0) = (s_0^2 + (x_0 * s_1^2))/2 \qquad (4)$$

$u(x_0)$=0.00345 (Type B)

4.1.2

Uncertainty from calibrants D, E, F and G

Calibrants D, E, F and G were obtained by appropriate dilution of the intermediate stock solution C with a concentration of 44.83 μg/L of Cr(VI), that is, first aspirating v_D, v_E, v_F and v_G of C by using pipetman (GILSON, USA) and then diluting to a v_6 of flask (SCHERF, Western Germany) to obtain calibrants D, E, F and G calibrants, respectively.

D: 0.134 μg/L, v_D:0.3mL, v_6:100mL
E: 0.224 μg/L, v_E:0.5mL, v_6:100mL
F: 0.448 μg/L, v_F:1.0mL, v_6:100mL
G: 0.672 μg/L, v_G:1.5mL, v_6:100mL

Final concentration of calibrants D, E, F and G are calculated by the following formula respectively:

$$c(D) = \frac{c(C) \times v_D}{v_6} \qquad (5)$$

$$c(E) = \frac{c(C) \times v_E}{v_6} \qquad (6)$$

$$c(F) = \frac{c(C) \times v_F}{v_6} \qquad (7)$$

$$c(G) = \frac{c(C) \times v_G}{v_6} \qquad (8)$$

4.1.2.1

Uncertainty from calibrants D, *u(c(D)*: (Final concentration: 0.134 μg/L, v_D=0.3mL, v_6=100mL, stock solution concentration *c(C)* = 44.83 μg/L)

In order to quantify the standard uncertainty of calibrant D, the quantification of uncertainty component of each volume are given in detail as follow:

Quantification of the uncertainty component of the volume of the 100 ml flask:

1) Calibration: The manufacturer SCHERF quotes a volume for the flask of 100±0.1 ml measured at a temperature of 20 C° and assuming a triangular distribution,

So the standard uncertainty (Type B):

$0.1ml/6^{1/2}=0.04ml$

2) Repeatability: the uncertainty due to variation in filling is estimated from a series of eleven fill and weigh experiments on a typical 100 ml flask gave standard deviation of 0.02 ml (Appendix 3 for original data). Hence, it is used directly as a standard uncertainty (Type A).

3) Temperature: according to the manufacturer SHCERF the flask has been calibrated at a temperature of 20 C°, whereas the laboratory temperature varies between the limits of ± 2 C°. The coefficient of volume expansion for water is $2.1*10^{-4}$ C^{o-1}, so the volume variation is:

$\pm(100*2*2.1*10^{-4}) = \pm 0.042ml$

Assuming a rectangular distribution for the temperature variation, therefore the standard uncertainty (Type B):

$0.042ml/3^{1/2}=0.024ml$

The three contribution are combined to give the standard uncertainty $u(v_6)$:

$u(v_6) = (0.04^2+0.02^2+0.024^2)^{1/2}=0.05$ ml

Quantification of the uncertainty component of the 0.3 ml pipetman:

Similarly calculate the calibration, repeatability and temperature effects.

1) Calibration: The manufacturer GILSON quotes a volume for the pipetman 0.200±0.003 ml measured at a temperature of 20 C° (Model P1000, re Appendix 4) which is very close to 0.300mL. So the standard uncertainty (Type B) is calculated assuming a triangular distribution.

$0.003ml/6^{1/2}=0.0012ml$

2) Repeatability: The manufacturer GILSON quotes a precision of less than 0.0006 mL (standard deviation) for the 0.200 mL pipetman at a temperature of 20 C° (Model P1000, re Appendix 4). Hence, it is used directly as a standard uncertainty (Type B).

3) Temperature: according to the manufacturer GILSON the pipetman has been calibrated at a temperature of 20 C°, whereas the laboratory temperature varies between

the limits of ± 2 C°. The uncertainty from this effect can be calculated from the estimate of the temperature range and the coefficient of the volume expansion. The volume expansion of the liquid is considerably larger than that of the pipetman so only the former needs to be considered. The coefficient of volume expansion for water is $2.1*10^{-4}$ $C^{\circ -1}$, which leads to a volume variation of

$\pm(0.3*2*2.1*10^{-4}) = \pm 0.00013$ml

The standard uncertainty (Type B) is calculated using the assumption of a rectangular distribution for the temperature variation, therefore

$0.00013\text{ml}/3^{1/2}=0.00007$ml

The three contribution are combined to give the standard uncertainty $u(v_D)$ of the volume

$u(v_D)=(0.0012^2+0.0006^2+0.00007^2)^{1/2}=0.0014$ ml

Using the results above, the uncertainty of calibrant D can be calculated from:

$$u(c(D)) = c(D) \times \sqrt{\frac{u(v_D)^2}{v_D^2} + \frac{u(v_6)^2}{v_6^2}} \qquad (9)$$

=0.00063 µg /L

4.1.2.2

Uncertainty from calibrants E, *u(c(E)*: (Final concentration: 0.224 µg/L, v_E=0.5mL, v_6=100mL, stock solution concentration *c(C)* = 44.83 µg/L)

Quantification of the uncertainty component of volume 100 mL flask are done similar to the previous section (re 4.1.2.1):

$u(v_6) = (0.04^2+0.02^2+0.024^2)^{1/2}=0.05$ ml

Quantification of the uncertainty component of the 0.5 ml pipetman:

Calibration: The manufacturer GILSON quotes a volume for the pipetman0.500±0.004 ml measured at a temperature of 20 C°(Model P1000, re Appendix 4), so for a volume 0.5 ml the standard uncertainty (Type B) is calculated assuming a triangular distribution as follow.

$0.004\text{ml}/6^{1/2}=0.0016$ml

Repeatability: The manufacturer GILSON quotes a precision of less than 0.001 mL (standard deviation) for the 0.500 mL pipetman at a temperature of 20 C° (Model P1000, re Appendix 4). Hence, it is used directly as a standard uncertainty (Type B).

Temperature: according to the manufacturer GILSON the pipetman has been calibrated at a temperature of 20 C°, whereas the laboratory temperature varies between the limits of ± 2 C°. The uncertainty from this effect can be calculated from the estimate of the temperature range and the coefficient of the volume expansion. The volume expansion of the liquid is considerably larger than that of the pipetman so only the former needs to be considered. The coefficient of volume expansion for water is $2.1*10^{-4}$ $C^{\circ -1}$, which leads to a volume variation

The standard uncertainty (Type B) is calculated using the assumption of a rectangular distribution for the temperature variation, therefore

$\pm(0.5*2*2.1*10^{-4})$ ml$/3^{1/2}$=0.000121ml

The three contributions are combined to give the standard uncertainty:

$u(v_E)=(0.0016^2+0.001^2+0.000121^2)^{1/2}$=0.0019 ml

Using the results above, the uncertainty of calibrant E can be calculated from:

$$u(c(E)) = c(E) \times \sqrt{\frac{u(v_E)^2}{v_E^2} + \frac{u(v_6)^2}{v_6^2}} \qquad (10)$$

=0.00086 µg /L

4.1.2.3

Uncertainty from calibrants F, *u(c(F)*: (Final concentration: 0.448 µg/L, v_F=1mL, v_6=100mL, stock solution concentration *c(C)* = 44.83 µg/L)

Quantification of the uncertainty component of the 1 ml pipetman:

Calibration: The manufacturer GILSON quotes a volume for the pipetman 1.000±0.008 ml measured at a temperature of 20 C°(Model P1000, re Appendix 4), so for a volume 1 ml the standard uncertainty (Type B) is calculated assuming a triangular distribution as follow.

0.008ml$/6^{1/2}$=0.0033ml

Repeatability: The manufacturer GILSON quotes a precision of less than 0.0015 mL (standard deviation) for the 1 mL pipetman at a temperature of 20 C° (Model P1000, re Appendix 4). Hence, it is used directly as a standard uncertainty (Type B).

Temperature: according to the manufacturer GILSON the pipetman has been calibrated at a temperature of 20 C°, whereas the laboratory temperature varies between the limits of ± 2 C°. The uncertainty from this effect can be calculated from the estimate

of the temperature range and the coefficient of the volume expansion. The volume expansion of the liquid is considerably larger than that of the pipetman so only the former needs to be considered. The coefficient of volume expansion for water is $2.1*10^{-4}$ $C^{\circ -1}$, which leads to a volume variation

The standard uncertainty (Type B) is calculated using the assumption of a rectangular distribution for the temperature variation, therefore

$\pm(1*2*2.1*10^{-4})$ ml$/3^{1/2}$=0.000242ml

The three contributions are combined to give the standard uncertainty:

$u(v_F)=(0.0033^2+0.0015^2+0.000242^2)^{1/2}$=0.0036 ml

Quantification of the uncertainty component of volume 100 mL flask is done similar to the previous section (re 4.1.2.1):

$u(v_6) = (0.04^2+0.02^2+0.024^2)^{1/2}$=0.05 ml

Using the results obtained, the uncertainty of calibrant can be calculated from:

$$u(c(F)) = c(F) \times \sqrt{\frac{u(v_F)^2}{v_F^2} + \frac{u(v_6)^2}{v_6^2}} \qquad (11)$$

=0.00163 µg /L

4.1.2.4

Uncertainty from calibrants G, *u(c(G)*: (Final concentration: 0.672 µg/L, v_G=1.5 mL, v_6=100 mL, stock solution concentration *c(C)* = 44.83 µg/L)

Quantification of the uncertainty component of volume of 100 mL flask is done similar to the previous section (re 4.1.2.1):

$u(v_6) = (0.04^2+0.02^2+0.024^2)^{1/2}$=0.05 ml

Quantification of the uncertainty component of the 1.5 ml pipetman:

Calibration: The manufacturer GILSON quotes a volume for the pipetman1.500±0.012 ml measured at a temperature of 20 C° (Model P5000, re Appendix 4), so for a volume 1.5 ml the standard uncertainty (Type B) is calculated assuming a triangular distribution as follow.

0.012ml$/6^{1/2}$=0.0049ml

Repeatability: The manufacturer GILSON quotes a precision of less than 0.004 mL (standard deviation) for the 0.200 mL pipetman at a temperature of 20 C° (Model P5000, re Appendix 4). Hence, it is used directly as a standard uncertainty (Type B).

Temperature: according to the manufacturer GILSON the pipetman has been calibrated at a temperature of 20 C°, whereas the laboratory temperature varies between the limits of ± 2 C°. The uncertainty from this effect can be calculated from the estimate of the temperature range and the coefficient of the volume expansion. The volume expansion of the liquid is considerably larger than that of the pipetman so only the former needs to be considered. The coefficient of volume expansion for water is $2.1*10^{-4}$ $C^{\circ -1}$, which leads to a volume variation

The standard uncertainty (Type B) is calculated using the assumption of a rectangular distribution for the temperature variation, therefore

$\pm(1.5*2*2.1*10^{-4})$ ml$/3^{1/2}$=0.000364ml

The three contributions are combined to give the standard uncertainty $u(v_G)$ of the volume

$u(v_G)=(0.0049^2+0.004^2+0.000364^2)^{1/2}$=0.0063 ml

Using the results above, the uncertainty of calibrant G can be calculated from:

$$u(c(G)) = c(G) \times \sqrt{\frac{u(v_G)^2}{v_G^2} + \frac{u(v_6)^2}{v_6^2}} \qquad (12)$$

=0.00284 μg /L

4.1.3 Quantification of uncertainty from sample concentration c_0

4.1.3.1 Calculation of weighted linear regression and T-test

Perform weighted linear regression to all calibrants and obtain values of *a* and *b* for the following formula:

$$x = a + b \times c \qquad (13)$$

1st step:

In Table 3, *w(D)* is calculated by the following expression:

$$w(D) = \frac{1}{u(D)^2} \qquad (14)$$

While $u(D)^2$ is calculated by the following expression:

$$u(D)^2 = \frac{s_0^2 + (s_1 \times x(D))^2}{2} \qquad (15)$$

w(E), w(F), w(G) and *w(H)* are calculated similarly.

Table 3

s_0	0,004	s_1	0,0278	Unit
	Abs1	Abs2	mean	
x(D)	0,0779	0,0809	0,0794	Abs
x(E)	0,1029	0,0973	0,1001	Abs
x(F)	0,1572	0,1551	0,1562	Abs
x(G)	0,2039	0,1989	0,2014	Abs
x(H)	0,0529	0,0411	0,0470	Abs
c(D)			0,134	$\mu g\ L^{-1}$
c(E)			0,224	$\mu g\ L^{-1}$
c(F)			0,448	$\mu g\ L^{-1}$
c(G)			0,672	$\mu g\ L^{-1}$
c(H)			0	$\mu g\ L^{-1}$
u(c(D))			0,00063	$\mu g\ L^{-1}$
u(c(E))			0,00086	$\mu g\ L^{-1}$
u(c(F))			0,00163	$\mu g\ L^{-1}$
u(c(G))			0,00284	$\mu g\ L^{-1}$
u(c(H))			0	$\mu g\ L^{-1}$
w(D)			95820,95319	Abs^{-2}
w(E)			84232,28652	Abs^{-2}
w(F)			57398,65747	Abs^{-2}
w(G)			42240,51715	Abs^{-2}
w(H)			112948,3825	Abs^{-2}
T1			392640,7968	Abs^{-2}
			12840,00773	$Abs^{-2}\ \mu g\ L^{-1}$
			18868,03218	$Abs^{-2}\ \mu g\ L^{-1}$
			25714,59855	$Abs^{-2}\ \mu g\ L^{-1}$
			28385,62752	$Abs^{-2}\ \mu g\ L^{-1}$
			0	$Abs^{-2}\ \mu g\ L^{-1}$
T2			85808,26598	$Abs^{-2}\ \mu g\ L^{-1}$
			1720,561035	$Abs^{-2}\ \mu g^2\ L^{-2}$
			4226,439208	$Abs^{-2}\ \mu g^2\ L^{-2}$
			11520,14015	$Abs^{-2}\ \mu g^2\ L^{-2}$
			19075,14169	$Abs^{-2}\ \mu g^2\ L^{-2}$
			0	$Abs^{-2}\ \mu g^2\ L^{-2}$
T3			36542,28209	$Abs^{-2}\ \mu g^2\ L^{-2}$
			7608,183683	Abs^{-1}
			8431,65188	Abs^{-1}
			8962,800364	Abs^{-1}
			8507,240153	Abs^{-1}
			5308,573976	Abs^{-1}
T4			38818,45006	Abs^{-1}
			1019,496614	$Abs^{-1}\mu g\ L^{-1}$
			1888,690021	$Abs^{-1}\mu g\ L^{-1}$
			4015,334563	$Abs^{-1}\mu g\ L^{-1}$
			5716,865383	$Abs^{-1}\mu g\ L^{-1}$
			0	$Abs^{-1}\mu g\ L^{-1}$
T5			12640,38658	$Abs^{-1}\mu g\ L^{-1}$

T_1, T_2, T_3, T_4 and T_5 are calculated by the following expressions:

$$T_1 = w(D) + w(E) + w(F) + w(G) + w(H) \quad (16)$$

$$T_2 = w(D) \times c(D) + w(E) \times c(E) + w(F) \times c(F) + w(G) \times c(G) + w(H) \times c(H) \quad (17)$$

$$T_3 = w(D) \times c(D)^2 + w(E) \times c(E)^2 + w(F) \times c(F)^2 + w(G) \times c(G)^2 + w(H) \times c(H)^2 \quad (18)$$

$$T_4 = w(D) \times x(D) + w(E) \times x(E) + w(F) \times x(F) + w(G) \times x(G) + w(H) \times x(H) \quad (19)$$

$$T_5 = w(D) \times c(D) \times x(D) + w(E) \times c(E) \times x(E) + w(F) \times c(F) \times x(F) + w(G) \times c(G) \times x(G) + w(H) \times c(H) \times x(H) \quad (20)$$

a and *b* values are obtained through the following expressions:

$$a = \frac{T_3 T_4 - T_2 T_5}{T_1 T_3 - T_2^2} = 0.0478 \ \text{Abs} \quad (21)$$

$$b = \frac{T_1 T_5 - T_2 T_4}{T_1 T_3 - T_2^2} = 0.2337 \ \text{L } \mu\text{g}^{-1} \ \text{Abs} \quad (22)$$

The Chi-squared test is employed for the significance test between the deviations of the estimated predictions and the standard uncertainties. T value is calculated by the following expression and listed in Table 4.

$$T = \sum_1^n \frac{(\text{exp}\, erimentalresult - predicted)^2}{(s\,\text{tan}\, dard.uncerta\,\text{int}\, y)^2} \quad (23)$$

T = 1.35

Table 4

s_0	s_1	n			a	b	x_0	c_0
0,004	0,0278	5			*0,0478*	*0,2337*	0,1001	0,224
	Abs1	Abs2	mean	Predicted x	Residual	Square(Residual)	Square (Combined Standard Uncertainty)	
x(D)	0,0779	0,0809	0,0794	0,079110035	0,000290	0,00000008	1,04361E-05	0,008056608
x(E)	0,1029	0,0973	0,1001	0,100140573	-0,000041	0,00000000	1,18719E-05	0,000138658
x(F)	0,1572	0,1551	0,1562	0,152483244	0,003667	0,00001345	1,7422E-05	0,771730511
x(G)	0,2039	0,1989	0,2014	0,204825916	-0,003426	0,00001174	2,3674E-05	0,49577279
x(H)	0,0529	0,0411	0,0470	0,047797901	-0,000798	0,00000064	8,8536E-06	0,071908096
					3 d.f.	n,s.	**T**	**1,347606664**

For 3 degrees of freedom, the upper critical value of T is 7.81 with a probability 0.05 and the lower level critical value of T is 0.35 with a probability 0.95, so the hypothesis is right which means the calibration curve is linear and values of *a* and *b* are acceptable.

2nd step:

When $u(D)^2$ is calculated by the following expression:

$$u(D)^2 = \frac{s_0^2 + (s_1 \times x(D))^2}{2} + (b \times u(C(D)))^2 \qquad (24)$$

$w(D)$ is recalculated by Equation (14). So Table 3 is recalculated and Table 5 is obtained:

Table 5

s_0	0,004	s_1	0,0278	Unit
	Abs1	Abs2	mean	
x(D)	0,0779	0,0809	0,0794	Abs
x(E)	0,1029	0,0973	0,1001	Abs
x(F)	0,1572	0,1551	0,1562	Abs
x(G)	0,2039	0,1989	0,2014	Abs
x(H)	0,0529	0,0411	0,0470	Abs
c(D)			0,134	$\mu g\ L^{-1}$
c(E)			0,224	$\mu g\ L^{-1}$
c(F)			0,448	$\mu g\ L^{-1}$
c(G)			0,672	$\mu g\ L^{-1}$
c(H)			0	$\mu g\ L^{-1}$
u(c(D))			0,00063	$\mu g\ L^{-1}$
u(c(E))			0,00086	$\mu g\ L^{-1}$
u(c(F))			0,00163	$\mu g\ L^{-1}$
u(c(G))			0,00284	$\mu g\ L^{-1}$
u(c(H))			0	$\mu g\ L^{-1}$
w(D)			95622,3353	Abs^{-2}
w(E)			83946,66143	Abs^{-2}
w(F)			56924,53141	Abs^{-2}
w(G)			41468,89306	Abs^{-2}
w(H)			112948,3825	Abs^{-2}
T1			390910,8037	Abs^{-2}
			12813,39293	$Abs^{-2}\ \mu g\ L^{-1}$
			18804,05216	$Abs^{-2}\ \mu g\ L^{-1}$
			25502,19007	$Abs^{-2}\ \mu g\ L^{-1}$
			27867,09614	$Abs^{-2}\ \mu g\ L^{-1}$
			0	$Abs^{-2}\ \mu g\ L^{-1}$
T2			84986,7313	$Abs^{-2}\ \mu g\ L^{-1}$
			1716,994653	$Abs^{-2}\ \mu g^2\ L^{-2}$
			4212,107684	$Abs^{-2}\ \mu g^2\ L^{-2}$
			11424,98115	$Abs^{-2}\ \mu g^2\ L^{-2}$
			18726,6886	$Abs^{-2}\ \mu g^2\ L^{-2}$
			0	$Abs^{-2}\ \mu g^2\ L^{-2}$
T3			36080,77209	$Abs^{-2}\ \mu g^2\ L^{-2}$
			7592,413423	Abs^{-1}
			8403,060809	Abs^{-1}
			8888,765579	Abs^{-1}
			8351,835063	Abs^{-1}
			5308,573976	Abs^{-1}
T4			38544,64885	Abs^{-1}
			1017,383399	$Abs^{-1}\mu g\ L^{-1}$
			1882,285621	$Abs^{-1}\mu g\ L^{-1}$
			3982,166979	$Abs^{-1}\mu g\ L^{-1}$
			5612,433162	$Abs^{-1}\mu g\ L^{-1}$
			0	$Abs^{-1}\mu g\ L^{-1}$
T5			12494,26916	$Abs^{-1}\mu g\ L^{-1}$

Similarly, values of a and b are obtained and T-test is conducted (Table 6).

Table 6

s_0	s_1	n			a	b	x_0	c_0
0,004	0,0278	5			*0,0478*	*0,2337*	0,1001	0,224
	Abs1	Abs2	mean	Predicted x	Residual	Square(Residual)	Square (Combined Standard Uncertainty)	
x(D)	0,0779	0,0809	0,0794	0,079108415	0,000292	0,00000009	1,04578E-05	0,008129995
x(E)	0,1029	0,0973	0,1001	0,100143072	-0,000043	0,00000000	1,19123E-05	0,000155736
x(F)	0,1572	0,1551	0,1562	0,152495996	0,003654	0,00001335	1,75671E-05	0,760041919
x(G)	0,2039	0,1989	0,2014	0,20484892	-0,003449	0,00001190	2,41145E-05	0,493274476
x(H)	0,0529	0,0411	0,0470	0,047790148	-0,000790	0,00000062	8,8536E-06	0,070517458
					3 d.f.	n,s.	**T**	**1,332119582**

With no significant change in T value in the above mentioned two steps, the uncertainty contribution from preparation of calibration solution is negligible. Finally *a* (0.0478 Abs) and *b* (0.2337 L μg^{-1}) are used for further calculation.

4.1.3.2 Calculation of uncertainty of sample concentration

Since the means of the sample reading x_0 is 0.1001 (0.1029, 0.0973), the concentration of sample can be calculated:

$$c_0 = (x_0 - a)/b = 0.224 \mu g / L$$

Calculating the uncertainty of c_o by the following formula (re *Accred. Qual. Assur.* 7(2002) 153-158)

$$u(c_0)^2 = \frac{1}{b^2}\left[u(x_0)^2 + \frac{T}{T_1(n-2)}\left(1 + \frac{(c_0 T_1 - T_2)^2}{T_1 T_3 - T_2^2}\right)\right] \qquad (25)$$

where

b=0.2337 L μg^{-1} Abs
$u(x_0)$=0.00345 Abs
T_1=390910,8037 Abs^{-2}
T_2=84986,7313 Abs^{-2} $\mu g\ L^{-1}$
T_3=36080,77209 Abs^{-2} $\mu g^2\ L^{-2}$
T=1,332119582
c_0=0.224 μg/L
n=5
So, $u(c_o)$=0.0154 μg/L

4.2 Uncertainty from f_h

Sample container was stirred for 1 minute to assure homogeneity before measurement (re 2.1.2). We will assume that heterogeneity has no significant influence on the results.

The verification of this assumption is done by duplicate sampling from the sample container (re Chapter 6).

4.3 Uncertainty from v_0, f_f, f_r, δ_w, f_e and f_a

Repeatability of sample/calibrant reading in the project is obtained from 6 times of repeated measurements of calibrants (blank and 0.448 ug/L Cr(VI) sample) which have gone through the same procedure (SI-LOV-AAS) before finally having the readouts (re 4.1.1).

That procedure (SI-LOV-AAS) can be seen as a black box. All the specified influences which we have analyzed before, including volume of sample (v_0), forms of Cr(VI) (f_f), derivatization (f_r), elution (f_e), washing (δ_w), ETAAS detection (f_a) and other factors we may have overlooked, are in fact combined together in causing the change of the readouts. So all those influences are the sources of repeatability of sample/calibrant reading. When we get the repeatability result of sample/calibrant reading we already have those uncertainty components in it.

The quantifications of all uncertainty components from v_0, f_f, f_r, δ_w, f_e and f_a are done as shown in the following.

4.3.1 Uncertainty from v_o

The volume has three major influences; calibration, repeatability and temperature effects.

Calibration: The manufacturer CAVRO (USA) quotes an inaccuracy less than 1% at full stroke for the syringe at a temperature of 20 C° (re Appendix 5). For 2 mL sample using a 5 mL of syringe pump the volume is 2±0.05 ml. The standard uncertainty (Type B) is calculated assuming a triangular distribution.

$$0.05\text{mL}/6^{1/2} = 0.0204\ \text{mL}$$

Repeatability: The manufacturer CAVRO (USA) quotes a imprecision of 0.05% CV within run at full stroke at a temperature of 20 C° (re Appendix 5). For 2mL of sample using 5 mL the precision is 0.0025 mL. Hence, it is used directly as a standard uncertainty (Type B).

Temperature: according to the manufacturer the syringe has been calibrated at a temperature of 20 C°, whereas the laboratory temperature varies between the limits of ± 2 C°. The uncertainty (Type B) from this effect can be calculated from the estimate of the temperature range and the coefficient of the volume expansion. The coefficient of volume expansion for water is $2.1*10^{-4}$ $C^{\circ -1}$, which leads to a volume variation of

$$\pm(2*2*2.1*10^{-4}) = \pm 0.00084\ \text{mL}$$

The standard uncertainty is calculated using the assumption of a rectangular distribution for the temperature variation, therefore

$0.00084 \text{ ml} / 3^{1/2} = 0.0005 \text{ mL}$

The three contributions are combined to give the standard uncertainty $u(v_0)$

$u(v_0) = (0.0204^2+0.0025^2+0.0005^2)^{1/2}=0.0206 \text{ mL}$

This uncertainty component will have the same contribution to both sample and calibrant.

4.3.2 Uncertainty from f_f

Samples are on-line adjusted to an acidity of 1.0 mol L^{-1} H^+ before loading. According to Appendix 5, the volume of acid delivered by the syringe of the pH adjustment pump has a coefficient of variation of less than 1%, which must be compounded with the spatial variation from possibly incomplete mixing.

After merging with the acid flow the sample passes through a 20 μL-column with beads of mean diameter 90μm which presumably improves homogeneity of acidity. We may safely assume that the spatial variability does not exceed 0.1 mol L^{-1} of H^+.

When the sample acidity ranges from 0.5 mol L^{-1} H^+ to 2.0 mol L^{-1} H^+, there is no significant change in the signals of the identical sample (re *Anal. Chem*. 77(205) 6032-6040). Therefore we can deduce the uncertainty from f_f is not significant. (Type B)

This uncertainty component will have the same contribution to both sample and calibrant.

4.3.3 Uncertainty from f_r

The derivatization reaction of Cr(VI) with DPC is strongly acid-dependent (re Chapter 2.2 expression (1)). From the analysis of Chapter 4.3.1, we may safely assume that the acid variability does not exceed 0.1 mol L^{-1} of H^+. In addition, the solution is in closed tubing system before loading on the sorbent material. The temperature in the lab is 20 ± 2 degrees. Therefore, uncertainty from f_r is probably small (Type B).

This uncertainty component will have the same contribution to both sample and calibrant.

4.3.4 Uncertainty from δ_w

The washing step is introduced after sample loading to reduce the introduction of matrix elements to the graphite tube of ETAAS which might cause significant influence in ETAAS measurement, because matrix elements in the last segment of sample solution (20μL) remain at the beads and relatively high amount of matrix elements in the sample make the absolute amount of matrix elements absorbed dominant.

Under the recommend operation parameters in the proposed method, the signals obtained for the calibrant containing 0.224 μg/L of Cr(VI) when no washing step is introduced (0 mL of washing solution is used) are 0.1000 and 0.0984 (abs) (mean 0.0992), while the signal for the identical calibrant with a washing step (0.5 mL of 0.5 M HCl of washing solution from carrier) are 0.1012 and 0.0979 (abs) (mean 0.0996). There is no significant difference whether washing step is introduced or not. This is because the prepared calibrant contains no matrix elements. The produced Cr(III) (re Equation (1)) is

not retained on surface of the beads due to the hydrophobic property of the sorbent. The produced Cr(III) goes to the waste directly with the solution which passes through the beads. Only the last segment of solution (equal to/less than the volume of sorbent volume 20μL) remains at the microcolumn which can be introduced to the ETAAS if no washing step is introduced. Most of the produced Cr(III) (more than 99%) goes to the waste with the solution. The error from the washing step is less than1% and therefore cannot be detected by this method of measurement.

This uncertainty component will have the same contribution to both sample and calibrant.

4.3.5 Uncertainty from f_e

Under the recommended operation parameters in the proposed method, the signals for a calibrant containing 0.7 μg/L of Cr(VI) are 0.1940 and 0.1912 (mean 0.1926abs) when 30 μL of eluent is used. The signals for the identical calibrant are 0.2075 and 0.1915 (mean 0.1985abs) when 40 μL of eluent is used. There is a 3% increase in absorbance when the volume of eluent increases by 10 μL. So, the increase of eluent volume can benefit the elution efficiency. Considering the capacity of graphite tube of ETAAS, more than 50μL of eluent normally is not recommended. Less volume of eluent can benefit a better enrichment factor. So finally 30 μL of eluent is chosen. The eluent is driven by the syringe pump which has less than 1% alteration in volume (re Appendix 5).

If assuming the sample (0.224 μg/L Cr(VI)) has the same performance as 0.7 μg/L of Cr(VI) calibrant and 10 μL of eluent more leads to 3% increase in the results (concentration of Cr(VI)). In addition, all the uncertain values are equal probable, Therefore we can calculate f_e (Type B) as following:

$$f_e = (1\% * 30\mu L/10\mu L) * 3\%/3^{1/2} = 0.00052$$

This uncertainty component will have the same contribution to both sample and calibrant.

4.3.6 Uncertainty from f_a

Duplicate analysis of pure chromium solutions by direct ETAAS resulted in the following results in Table 7. The uncertainty of each result was estimated from Equation (3) and a T-test was made to test the significance of contributions from all other uncertainty components including v_0, f_f, f_r, δ_w and f_e.

Table 7

Conc. μg/L	Abs1	*u(x)*	Abs2	*u(x)*	*T*
0	0,0012	0.004	0,0011	0.004	0.000312
5	0,0332	0.004105	0,0336	0.004108	0.004744
10	0,0660	0.004401	0,0663	0,004404	0.002322
20	0,1478	0.005734	0,1416	0.005612	0.597094
				SUM	0.604473
				d.f.	4
				p	0.962565

Being just barely significant at the 5% level, we may conclude that the uncertainty from f_a is dominant and that the combined contributions from v_0, f_f, f_r, δ_w and f_e are small in comparison.

In conclusion, we have therefore not overlooked any significant source of uncertainty.

4.4 Uncertainty from δi

For 0.5 µg/L of Cr(VI), ratio of Mo(IV)/Cd(II)/Cu(II) to Cr(VI) <1000 causes less than10 % interference level; ratio of Cr(III)/Cr(VI) ratio<15 causes less than 10 % interference level (re *Anal. Chem*. 77(205) 6032-6040).

If assuming that quantities of Mo, Cd, and Cu are expressed in µg/L, then it can be deduced that up to 500 µg/L of Mo+Cd+Cu give rise to an interference value of Cr(VI) of δ_1 <0.05 µg/L.

Let us moreover assume that the sum of concentrations of Mo, Cd, and Cu rarely exceed 50 µg/L, then the interference δ_1 <0.005 µg/L.

To estimate its uncertainty, let us assume that all values between zero and 50 µg/L are equally probable:

$$u(\delta_1) < 0{,}005/\sqrt{3} = 0{,}003 \text{ µg/L}$$

Similarly we may deduce that 7.5 µg/L of Cr (III) creates an interference of < 0.05 µg/L; if we moreover assume that Cr(III) rarely exceed 1 µg/L, we can obtain an interference uncertainty of

$$u(\delta_2) \le \frac{0.05}{7.5\sqrt{3}} = 0.004 \quad \text{µg/L}$$

The combined uncertainty from interference for the 2 mL of sample with a concentration of 0.5µg/L of Cr(VI):

$$u(\delta_i) < (0.003^2+0.004^2)^{0.5} * 2 \text{ mL} * 10^{-3}$$

So $u(\delta_i) < 0.00001$µg (Type B)

Although the above mentioned result is obtained from 0.5 µg/L of Cr(VI), the interference has no relationship with the concentration of Cr(VI). So for the sample in the project with 0.224µg/L of Cr(VI), the contribution to the combined uncertainty is the same.

Since the Cr(III) can be detected by ETAAS without discrimination from Cr(VI), a washing step is introduced to wash away Cr(III) which is possible weakly retained on the beads by surface adsorption. Because Cr(III) in the sample does not reacted with DPC, that adsorption is the only way for Cr(III) to be retained on the beads. From the above mentioned analysis, when the concentration of Cr(III) is less than 1 µg/L and a washing step is employed, the weakly retained Cr(III) can be washed away and the uncertainty from Cr(III) is less than 0.004 µg/L.

4.5 Traceability

Original stock solution A is from Merck and has a concentration of 1000±2mg/L of chromate (K_2CrO_4, Merck, Order nr.: 1197800500, Batch nr.: 90361942, with Certificate of Analysis (re Appendix 2)). The date of starting use is Dec. 5, 2000.

The stock solution is guaranteed to be valid for three years, we assume it can be valid at least 10 years. The stability of the chromate stock solution ought to be checked by another chromate standard solution which is prepared from ultra pure K_2CrO_4 salt.

All the calibrants are prepared from intermediate stock solution C, which was prepared from stock solution A. The calculation of concentration (Traceability T_r) is as following:

$$c(C) = T_r = \frac{c(A) \times v_1 \times v_3}{v_2 \times v_4} \qquad (26)$$

Quantification of the uncertainty of the concentration of intermediate stock solution C, *u(c(C),* is done as shown in the following:

$v_1 = v_3 = 1$ mL

$v_2 = v_4 = 100$ mL

Concentration in Cr(VI) of stock solution A: $c(A) = 448.3$ mg/L

$$T_r = \frac{448.3 \times 10^3 \times 1 \times 1}{100 \times 100} = 44.83 \mu g / L$$

2 mg/L of chromate is equal to 0.8966 mg/L of Cr(VI) and is directly used as the uncertainty of the concentration of stock solution A (re Appendix 2).

Quantification of the uncertainty component of the 1 ml pipetman (re 4.1.2.3):

$u(v_1) = u(v_3) = (0.0033^2+0.0015^2+0.000242^2)^{1/2} = 0.0036$ mL

Quantification of the uncertainty component of volume 100 mL flask is done similar to the previous section (re 4.1.2.1):

$u(v_2) = u(v_4) = (0.04^2+0.02^2+0.024^2)^{1/2}=0.05$ mL

Using the above results, the uncertainty $u(T_r)$ of concentration of intermediate stock solution C can be calculated from:

$$u(T_r) = c(C) \times \sqrt{\frac{u(c(A))^2}{c(A)^2} + \frac{u(v_1)^2}{v_1^2} + \frac{u(v_2)^2}{v_2} + \frac{u(v_3)^2}{v_3} + \frac{u(v_4)^2}{v_4}} \qquad (27)$$

=0.2473 μg /L

5 Calculation of combined uncertainty

The measurand is given by Equation (2):

$$m_0 = c_0 \times v_0 \times f_h \times f_f \times f_r \times f_e \times f_a \times 10^{-3} + \delta_i + \delta_w \qquad (2)$$

For the sample which has the readings 0.1029Abs and 0.0973Abs, the concentration c_o can be calculated by the calibration curve $x = a+b*c$ (a = 0.0478Abs, b = 0.2337 L μg^{-1}) (re Chapter 4.1.31). So c_o = 0.224 μg/L.

Other intermediate values are listed in Table 8. Employing those values:

m_0 = 0.224*2*1*1*1*1*1*10^{-3}+0+0= 0.000448 μg = 0.448 ng.

Table 8 Intermediate values and uncertainties

	Description	Value	Paragraph no.	Standard uncertainty *u(x)*	Relative standard uncertainty *u(x)/x*
c_0	Concentration of Cr(VI) in the sample	0.224 μg/L	Chapter 4.1	0.0154 μg/L	0.069
v_0	Volume of sample	2 mL	Chapter 4.3.1	0.0206 mL	Included in the repeatability
f_h	Influence of heterogeneity of sample	1.0	Chapter 4.2	unknown	≈0
f_f	Influence of forms of Cr(VI) in the sample before loading	1.0	Chapter 4.3.2	unknown	Included in the repeatability
f_r	Influence of the derivatization of Cr(VI)	1.0	Chapter 4.3.3	unknown	Included in the repeatability
f_e	Inlfuence of elution	1.0	Chapter 4.3.5	0.00052	Included in the repeatability
f_a	Influence of ETAAS detection	1.0	Chapter 4.3.6	repeatablility	Included in the repeatability
δ_i	Total influence of interferences ions	0 μg	Chapter 4.4	0.00001 μg	∝
δ_w	Influence of washing step	0 μg	Chapter 4.3.4	Included in the repeatability	
T_r	Traceability	44.83 μg/L	Chapter 4.5	0.2473 μg/L	0.0055

Based on Equation (2), we can obtain the combined uncertainty of measurand

$$u(m_0)^2 = m_0^2 * \left(\left(\frac{u(c_0)}{c_0} \right)^2 + \left(\frac{u(v_0)}{v_0} \right)^2 + \left(\frac{u(T_r)}{T_r} \right)^2 + \left(\frac{u(f_h)}{f_h} \right) + \left(\frac{u(f_f)}{f_f} \right) + \left(\frac{u(f_r)}{f_r} \right) + \left(\frac{u(f_e)}{f_e} \right) + \left(\frac{u(f_a)}{f_a} \right) \right)^2$$
$$+ u(\delta_i)^2 + u(\delta_w)^2 \qquad (28)$$

The uncertainty component from the sample concentration, $u(c_o)$ = 0.0154 μg/L (re Chapter 4.1.3.2)

In the proposed project, sample and all calibrants experience the same procedure from the introduction of 2 mL of solution(sample or calibrants) to the pretreatment system to the atomization of ETAAS before the final signals are obtained. Factors including volume of sample (v_0), forms of Cr(VI) (f_f), derivatization (f_r), elution (f_e), washing (δ_w), ETAAS detection (f_a) have the same contribution to both sample and calibrants. All those factors are combined together and influence the readouts, so repeatability of reading already contains the uncertainty components from $v_0, f_f, f_r, \delta_w, f_e$ and f_a (re Chapter 4.3) and is included in the uncertainty of sample concentration (re Equation (25)).

The homogeneity of solution is verified by duplicate sampling from the sample container (re Chapter 6). The uncertainty from homogeneity is assumed to be zero after thorough mixing by shaking. So $u(f_h) = 0, f_h = 1$.

Uncertainty component of interference $u(\delta_i)$ = 0.00001μg = 0.01 ng (re Chapter 4.4)

The intermediate stock solution C is made from commercial chromate stock solution A by proper dilution. The uncertainty component from traceability in the preparation of the intermediate stock solution C which has a concentration of 44.83 μg/L ($c(C)$) is 0.2473 μg/L (re Chapter 4.5).

All the intermediate standard uncertainties are collected in Table 9. Therefore, the combined uncertainty for the measurand is:

$$u(m_0)^2 = m_0^2 * \left(\left(\frac{u(c_0)}{c_0} \right)^2 + \left(\frac{u(T_r)}{T_r} \right)^2 + \left(\frac{u(f_h)}{f_h} \right) \right)^2 + u(\delta_i)^2 \qquad (29)$$

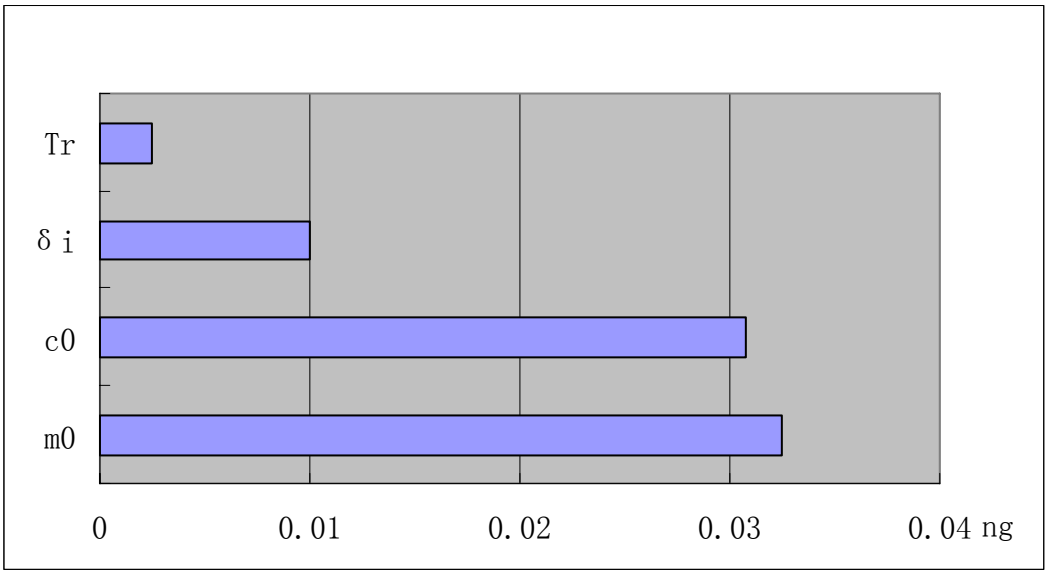
$$= 0.000448^2 * \left(\left(\frac{0.0154}{0.224} \right)^2 + \left(\frac{0.2473}{44.83} \right)^2 + \left(\frac{0}{1} \right)^2 \right) + 0.00001^2$$

$u(m_0)$ = 0.000032 μg = 0.032 ng

Therefore, the combined standard uncertainty for the 2 mL of sample contains 0.000448 μg of chromium is 0.000032 μg.

The uncertainty contributions are shown in a diagram (Fig. 3). As seen from the figure, the uncertainty from the c_o is the main component.

Fig. 3 Uncertainty contribution



6 Verification of the uncertainty budget

Verification of homogeneity of sample solution is done as described in the following sequence: measurement of sample solution from the first time sampling (0.0973Abs), blank check (use blank instead of sample, 0.04160Abs, 0.0486Abs), measurement of calibrants (0.448μg/L, 0.1551Abs, 0.1626ABs), measurement of sample solution from the second sampling (0.1053Abs). A T-test was made to test the influence of heterogeneity based on Equation (3) (re Chapter 4.1.1)

$$u^2(x) = s_0^2 + (x * s_1^2) \quad (3)$$

where

$s_0 = 0.0040$
$s_1 = 0.0278$

Appendix 1 Symbols and abbreviations used in the report

Symbol	definition	unit
a	intercept of the calibration curve $x=a+bc$	no unit (Abs)
b	slope of the calibration curve $x=a+bc$	L μg^{-1}
c_0	Concentrations of Cr(VI) in the sample	$\mu g\ L^{-1}$
c	Concentrations of Cr(VI) in sample or calibrants	$\mu g\ L^{-1}$
A	Original stock solution	No unit
B	Intermediate stock solution B	No unit
C	Intermediate stock solution C	No unit
D	Calibrant D	No unit
E	Calibrant E	No unit
F	Calibrant F	No unit
G	Calibrant G	No unit
H	Calibrant H	No unit
$c(A)$	Concentration of Cr(VI) in the original stock solution, 1000 mg L^- of chromate	mg L^{-1}
$c(C)$	Concentration of Cr(VI) in the stock solution C, 44.83 µg L^{-1} of Cr(VI)	$\mu g\ L^{-1}$
$c(D)$	Concentration of Cr(VI) in the calibrant D	$\mu g\ L^{-1}$
$c(E)$	Concentration of Cr(VI) in the calibrant E	$\mu g\ L^{-1}$
$c(F)$	Concentration of Cr(VI) in the calibrant F	$\mu g\ L^{-1}$
$c(G)$	Concentration of Cr(VI) in the calibrant G	$\mu g\ L^{-1}$
$c(H)$	Concentration of Cr(VI) in the calibrant H	$\mu g\ L^{-1}$
f_h	influence of heterogeneity of sample	factor, no unit
f_f	influence of the forms of Cr(VI) in the sample right before loading	factor, no unit
f_r	influence of the derivatization of Cr(VI)	factor, no unit
δ_1	influence of interference ions including Mo, Cd, Cu	$\mu g\ L^{-1}$
δ_2	influence of interference ions including Cr(III)	$\mu g\ L^{-1}$
δ_i	Total influence of interference ions including Mo, Cd, Cu, Cr(III) etc in 2 mL sample.	µg
δ_w	influence of washing step after sample loading on the column	µg
f_e	influence of elution	factor, no unit
f_a	influence of running ETAAS temperature program	abs
m_0	Measurand, total mass of soluble hexavalent chromium (CrO_4^{2-} and $Cr_2O_7^{2-}$) in ca. 2 mL (accurately measured volume) of sample at the time of being taken from a container with ca. 1 liter of pre-filtered (by 0.45 µm pore size membrane) environmental water sample,	µg
n	Number of calibrants	Integer
s_0	Represents a constant contribution to the overall uncertainty in the expression $u(x)^2=s_0^2+(s_1*x)^2$ which documenting uncertainty dependent on absorbance level	Abs
s_1	Proportionality constant in the expression $u(x)^2=s_0^2+(s_1*x)^2$ which documenting uncertainty dependent on absorbance level	No unit

T_1	Sum(re expression (19))	Abs^{-2}
T_2	Sum(re expression (20))	Abs^{-2} µg L^{-1}
T_3	Sum(re expression (21))	Abs^{-2} $µg^2$ L^{-2}
T_4	Sum(re expression (22)	Abs^{-1}
T_5	Sum(re expression (23))	Abs^{-1}µg L^{-1}
T	Sum, statistic	No unit
T_r	Traceability	µg L^{-1}
$u(c(C))$	Uncertainty of concentration of stock solution C	µg L^{-1}
$u(c(D))$	Uncertainty of concentration of calibrant D	µg L^{-1}
$u(c(E))$	Uncertainty of concentration of calibrant E	µg L^{-1}
$u(c(F))$	Uncertainty of concentration of calibrant F	µg L^{-1}
$u(c(G))$	Uncertainty of concentration of calibrant G	µg L^{-1}
$u(c(H))$	Uncertainty of concentration of calibrant H	µg L^{-1}
$u(c(C))$	Uncertainty of concentration of stock solution C	µg L^{-1}
$u(c_0)$	Uncertainty of the concentration of sample	µg L^{-1}
$u(x(D))$	Uncertainty of calibrant D reading	Abs
$u(x(E))$	Uncertainty of calibrant E reading	Abs
$u(x(F))$	Uncertainty of calibrant F reading	Abs
$u(x(G))$	Uncertainty of calibrant G reading	Abs
$u(x(H))$	Uncertainty of calibrant H reading	Abs
$u(D)$	Combined uncertainty of $u(c(D))$ and $u(x(D))$	Abs
$u(E)$	Combined uncertainty of $u(c(E))$ and $u(x(E))$	Abs
$u(F)$	Combined uncertainty of $u(c(F))$ and $u(x(F))$	Abs
$u(G)$	Combined uncertainty of $u(c(G))$ and $u(x(G))$	Abs
$u(H)$	Combined uncertainty of $u(c(H))$ and $u(x(H))$	Abs
$u(T_r)$	Uncertainty of traceability	µg L^{-1}
$u(m_0)$	Uncertainty of the measurand	µg
$u(x)$	Uncertainty of absorbance x	Abs
$u(x_0)$	Uncertainty of sample reading	Abs
$u(v_0)$	Uncertainty of volume v_0	mL
$u(v_1)$	Uncertainty of volume v_1	mL
$u(v_2)$	Uncertainty of volume v_2	mL
$u(v_3)$	Uncertainty of volume v_3	mL
$u(v_4)$	Uncertainty of volume v_4	mL
$u(v_6)$	Uncertainty of volume v_6	mL
$u(v_D)$	Uncertainty of volume v_D	mL
$u(v_E)$	Uncertainty of volume v_E	mL
$u(v_F)$	Uncertainty of volume v_F	mL
$u(v_G)$	Uncertainty of volume v_G	mL
$u(\delta_1)$	Uncertainty of δ_1	µg L^{-1}
$u(\delta_2)$	Uncertainty of δ_2	µg L^{-1}
$u(\delta_i)$	Uncertainty of δ_i	µg
$u(\delta_w)$	Uncertainty of δ_w	µg
$u(f_h)$	Uncertainty of f_h	No unit
$u(f_f)$	Uncertainty of f_f	No unit
$u(f_r)$	Uncertainty of f_r	No unit
$u(f_h)$	Uncertainty of f_h	No unit

$u(f_e)$	Uncertainty of f_e	No unit
$u(f_a)$	Uncertainty of f_a	No unit
v_0	sample volume (ca. 2 mL) aspirated by a high precision syringe pump controlled by step-motor and computer	mL
v_1	Volume of stock solution A for the preparation of stock solution B	mL
v_2	Volume of stock solution B	mL
v_3	Volume of stock solution B for the preparation of stock solution C	mL
v_4	Volume of stock solution C	mL
v_6	Volume of stock solution for the preparation of all calibrants	mL
v_C	Volume of A for the preparation of stock solution C	mL
v_D	Volume of C for the preparation of calibrant D	mL
v_E	Volume of C for the preparation of calibrant E	mL
v_F	Volume of C for the preparation of calibrant F	mL
v_G	Volume of C for the preparation of calibrant G	mL
x	Absorbance reading obtained from sample or calibrants	Abs, no unit
x_0	Absorbance reading obtained from the sample	Abs, no unit
$x(D)$	Absorbance reading obtained from calibrant D	Abs, no unit
$x(E)$	Absorbance reading obtained from calibrant E	Abs, no unit
$x(F)$	Absorbance reading obtained from calibrant F	Abs, no unit
$x(G)$	Absorbance reading obtained from calibrant G	Abs, no unit
$x(H)$	Absorbance reading obtained from calibrant H	Abs, no unit
$w(D)$	Weighting factor of calibrant D for performing weighted linear regression	Abs^{-2}, no unit
$w(E)$	Weighting factor of calibrant E for performing weighted linear regression	Abs^{-2}, no unit
$w(F)$	Weighting factor of calibrant F for performing weighted linear regression	Abs^{-2}, no unit
$w(G)$	Weighting factor of calibrant G for performing weighted linear regression	Abs^{-2}, no unit
$w(H)$	Weighting factor of calibrant H for performing weighted linear regression	Abs^{-2}, no unit
z	Percentage of chromium presented as chromate	No unit

Appendix 2

Certificate of Analysis

MERCK

http://certificates.merck.de

Date of print: *24.04.2006*

1.19780.0500 Chromate standard solution (potassium chromate in water) 1000 mg/l CrO_4^{2-}

Batch 90361942

		Batch Values	
Concentration	β (CrO_4^{2-})	1000	mg/l

Determination method: Iodometric titration.
(traceable to NIST - SRM 136e)
Accuracy of the method: +/- 2 mg

Test date: *07.05.1999*
Minimum shelf life: *31.05.2002*

Wolfgang Gernand

Analytical laboratory

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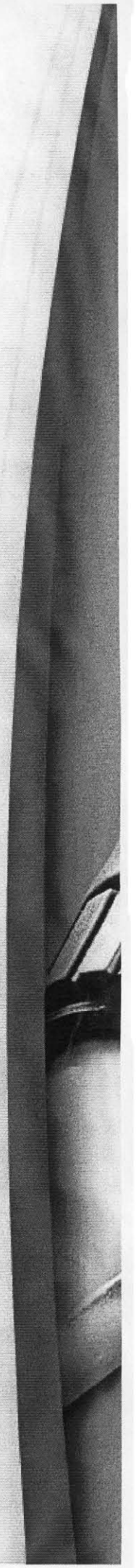
Appendix 3 Repeatability experiments of 100 mL flask (20 C°, density of water: 0.998203 g/cm3)

n	1	2	3	4	5	6
Weight(g)	99.8346	99.7987	99.7978	99.7967	99.7931	99.8316
volume(cm3)	100.01	99.98	99.98	99.98	99.97	100.01
n	7	8	9	10	11	STD
Weight(g)	99.8444	99.8463	99.7877	99.7969	99.8336	
volume(cm3)	100.02	100.03	99.97	99.98	100.01	0.02

Appendix 4 Speciation of GILSON pipetman

PIPETMAN P Range of Models

Model (Diamond Tips)	Volume (µl)	Accuracy (systematic error)		Precision (random error)		Model	Reference Number
		Absolute µl	Relative %	Absolute S.D. µl	Relative S.D. %		
P2 (D10, DL10)	Min. 0.2	± 0.024	± 12	≤ 0.012	≤ 6	P2	F144801
	0.5	± 0.025	± 5	≤ 0.012	≤ 2.50		
	Max. 2	± 0.030	± 1.5	≤ 0.014	≤ 0.70		
P10 (D10, DL10)	Min. 1	± 0.025	± 2.5	≤ 0.012	≤ 1.25	P10	F144802
	5	± 0.075	± 1.5	≤ 0.030	≤ 0.60		
	Max. 10	± 0.1	± 1	≤ 0.040	≤ 0.40		
P20 (D200)	Min. 2	± 0.1	± 5.0	≤ 0.03	≤ 1.50	P20	F123600
	5	± 0.1	± 2.0	≤ 0.04	≤ 0.80		
	10	± 0.1	± 1.0	≤ 0.05	≤ 0.50		
	Max. 20	± 0.2	± 1.0	≤ 0.06	≤ 0.30		
P100 (D200)	Min. 20	± 0.35	± 1.8	≤ 0.10	≤ 0.50	P100	F123615
	50	± 0.4	± 0.8	≤ 0.12	≤ 0.24		
	Max. 100	± 0.8	± 0.8	≤ 0.15	≤ 0.15		
P200 (D200)	Min. 50	± 0.5	± 1	≤ 0.20	≤ 0.4	P200	F123601
	100	± 0.8	± 0.8	≤ 0.25	≤ 0.25		
	Max. 200	± 1.6	± 0.8	≤ 0.30	≤ 0.15		
P1000 (D1000)	Min. 200	± 3.0	± 1.5	≤ 0.6	≤ 0.30	P1000	F123602
	500	± 4.0	± 0.8	≤ 1.0	≤ 0.20		
	Max. 1000	± 8.0	± 0.8	≤ 1.5	≤ 0.15		
P5000 (D5000)	Min. 1000	± 12	± 1.2	≤ 3.0	≤ 0.30	P5000	F123603
	2000	± 12	± 0.6	≤ 5.0	≤ 0.25		
	Max. 5000	± 30	± 0.6	≤ 8.0	≤ 0.16		
P10ml (D10ml)	Min. 1 ml	± 30	± 3	≤ 6	≤ 0.6	P10ml	F161201
	2 ml	± 30	± 1.5	≤ 6	≤ 0.3		
	5 ml	± 40	± 0.8	≤ 10	≤ 0.2		
	Max. 10 ml	± 60	± 0.6	≤ 6	≤ 0.16		



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7 - Specifications

A. Dimensions

Height	10.00 in	(25.4 cm)
Width	2.56 in	(6.5 cm)
Depth	5.61 in	(14.2 cm) from valve to board connector
Weight	4.5 lbs.	(2.0 kg)

B. Power Requirements

	Supply Voltage	Current
Peak	24 VDC ±10%	850 mA
Holding	24 VDC ±10%	200 mA

C. Resolution

Standard Pump	3,000/12,000 steps
High Resolution Pump	3,000/24,000 steps

D. Plunger Drive

Principle	Stepper motor driven lead screw with optical feedback
Travel	60 mm

E. Plunger Speeds

0.8 Second-10min/stroke for standard resolution pump
1.5 Seconds-20 min/stroke for high resolution pump
(Speed ranges vary depending on the syringe size and tubing.)

F. Syringes

Sizes	Aspirate:	50 uL – 1.0 ml
	Reagent:	500 uL – 25.0 ml
Material	Barrel:	Borosilicate Glass
	Plunger:	Stainless Steel
	Seal:	Virgin Teflon

G. Imprecision

0.05% CV within run at full stroke

H. Inaccuracy

<1% at full stroke

Paper I

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Evaluation of a novel PTFE material for use as a means for separation and preconcentration of trace levels of metal ions in sequential injection (SI) and sequential injection lab-on-valve (SI-LOV) systems Determination of cadmium(II) with detection by electrothermal atomic absorption spectrometry (ETAAS)

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Abstract

The operational characteristics of a novel poly(tetrafluoroethylene) (PTFE) bead material, granular Algoflon®, used for separation and preconcentration of metal ions via adsorption of on-line generated non-charged metal complexes, were evaluated in a sequential injection (SI) system furnished with an external packed column and in a sequential injection lab-on-valve (SI-LOV) system. Employed for the determination of cadmium(II), complexed with diethyldithiophosphate (DDPA), and detection by electrothermal atomic absorption spectrometry (ETAAS), its performance was compared to that of a previously used material, Aldrich PTFE, which had demonstrated that PTFE was the most promising for solid-state pretreatments. By comparing the two materials, the Algoflon® beads exhibited much higher sensitivity (1.6107 $\mu g\,l^{-1}$ versus 0.2956 $\mu g\,l^{-1}$ per integrated absorbance (s)), and better retention efficiency (82% versus 74%) and enrichment factor (20.8 versus 17.2), although a slightly smaller linear dynamic range (0.05–0.25 $\mu g\,l^{-1}$ versus 0.05–1.00 $\mu g\,l^{-1}$). Moreover, no flow resistance was encountered under the experimental conditions used. The results obtained on three standard reference materials were in good agreement with the certified values.

Keywords: Sequential injection (SI); On-line separation and preconcentration; Sequential injection lab-on-valve (SI-LOV); Electrothermal atomic absorption spectrometry (ETAAS); Renewable column; Packed column; Cd.

1. Introduction

Nowadays, electrothermal atomic absorption spectrometry (ETAAS) is one of the most powerful and popular analytical tools for the determination of ultratrace levels of elements [1–3] because it generally offers sufficient sensitivity and selectivity. Nevertheless, it is potentially prone to spectroscopic and/or non-spectroscopic interferences, the latter type being associated with the composition of the sample matrix, an especially serious source being the presence of high levels of salts. Various schemes have been suggested to alleviate the interfering effects and facilitate reliable analyses, such protocols ranging from instrument modifications (e.g., background correction) to experimental designs (e.g., standard addition or internal standardisation). However, instead of implementing such approaches, there is a much simpler and effective solution to the problem, namely to subject the sample to appropriate pretreatments before it is presented to the detector, that is, separating the analyte from the matrix and then at the

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same time accomplishing analyte preconcentration – which might, indeed, be advantageous/necessary if very small concentrations are to be measured in order to bring the analyte concentration within the dynamic range of the instrument.

The new generations of flow injection (FI), that is, sequential injection (SI), and SI-lab-on-valve (SI-LOV), have proven to entail a number of advantages for executing such separation and preconcentration techniques on-line in comparison with conventional batch pretreatment techniques, which normally are labor intensive, time consuming, and utilize large amounts of sample and reagents resulting in a substantial generation of waste materials [4,5].

Separation and preconcentration SI-procedures employing liquid–liquid extraction [4,6,7], hydride generation [8], and solid phase extraction [6,9–16] are areas of increasing interest. However, among the various approaches potentially feasible to effect matrix elimination and preconcentration of analyte, methods based on solid phase extraction appear to be the most efficient and attractive ones.

Sorbent materials used in solid phase extraction fall into two categories: hydrophilic, e.g., ion-exchange resins [17], and hydrophobic, e.g., poly(tetrafluoroethylene) (PTFE) or octadecyl-chemically modified poly(styrenedivinylbenzene) copolymers (C18-PS/DVB) [18], which have been applied for the determination of metal ions down to the sub $ng\,l^{-1}$ level. A condition for handling these types of materials in the LOV format – which is based on a renewable scheme, where the solid phase in the form of a packed column of beads is renewed for each analytical cycle – is that the beads applied possess the features of being perfectly spherical, uniform in size, non-compressible in the suspending solution, and preferentially having a density similar to that of water.

As compared to hydrophilic bead materials, hydrophobic beads, which implicitly can retain only non-charged compounds, are potentially much more versatile and interesting. Firstly, because they do not rely on the presence of specific functional groups on the beads, which might be lost or deactivated by irreversible interference. For the same reasons, such materials are, therefore, advantageously used in the LOV format. And secondly, and most importantly, because one can by intelligent selection of the chelating reagent obtain increased selectivity via generation of the non-charged complex to be adsorbed on the surface of the hydrophobic surface. And one can, again by exploiting good chemistry, obtain higher tolerances for potentially interfering ions, plus eliminate inert ions in samples of high salt contents.

Hydrophobic sorbents have been extensively employed for adsorption of neutral complexes formed from transition metals and chelating reagents. In a previous work from this group [18], the feasibility of the bead-injection/elution SI-LOV approach for handling hydrophobic PTFE beads as a renewable column material for separation and preconcentration of trace metal ions by ETAAS was assessed. The PTFE beads used showed much better performance as compared with the other hydrophobic material tested, C18-PS/DVB. Thus, the limit of detection was enhanced more than 20-fold. Furthermore, the enrichment factor and the retention efficiency were increased by more than twice, nearly three-fold, from 7.4 to 17.2 and from 28 to 74%, respectively.

However, the inherent characteristics of the PTFE beads make this material difficult to manipulate in an on-line system, especially as compared to the easy-to-handle hydrophilic sorbents. Bearing in mind the physical properties desired of the bead materials, such as morphology and density, the PTFE beads used appeared far from ideal. Thus, under a microscope they emerged more like lumps of irregular shape, and were (despite initial sieving) of very non-uniform size. In operation in the integrated microconduits this led to tight packing of the columns, resulting in increased flow resistance together with incomplete transportation of the loaded beads from one microcolumn to the other within the LOV, where appropriate suspensions are a must to facilitate the smooth manipulation of the beads. Since the density of PTFE (viz., $2.1\,g\,ml^{-1}$) is much higher than that of the suspending solvent used (ethanol), the beads tended to settle down quickly which, in turn, caused poorer reproducibility for manipulating and transferring them within the microconduit channels. Attempts have, therefore, been made to locate PTFE bead of perfect spherical shape and size homogeneity in order to exploit them in the LOV approach.

After a thorough search we have come upon a material which, although not entirely ideal for our purposes, might serve as a potentially serious candidate, because it appears to have the right morphology. This material is named Granular PTFE Algoflon®. Since it is spherical and possesses higher hydrophobicity than the previously used PTFE bead material, improvements in both the physical and the analytical performances would be expected to be achieved.

In the present research, the performance of the granular PTFE beads is examined and discussed by applying them in SI and SI-LOV systems for the determination of trace amount of cadmium environmental samples of complex matrices. Cadmium was selected as the model analyte because it is known to be a highly toxic metal, which plays important roles in the biological metabolism.

2. Experimental

2.1. Instrumentation

An atomic absorption spectrometer (PerkinElmer, model AAnalyst 600) with Zeeman background correction accommodating a transversely heated pyrolytically coated graphite tube (PerkinElmer, part no. B3000641), and an autosampler (PerkinElmer AS-800) were employed. The cadmium hollow cathode lamp (S&J Juniper & Co., Harlow, Essex, UK) was operated at 5 mA, using a wavelength of 228.8 nm and a spectral bandpass of 0.7 nm. The results were obtained by the integrated peak area mode.

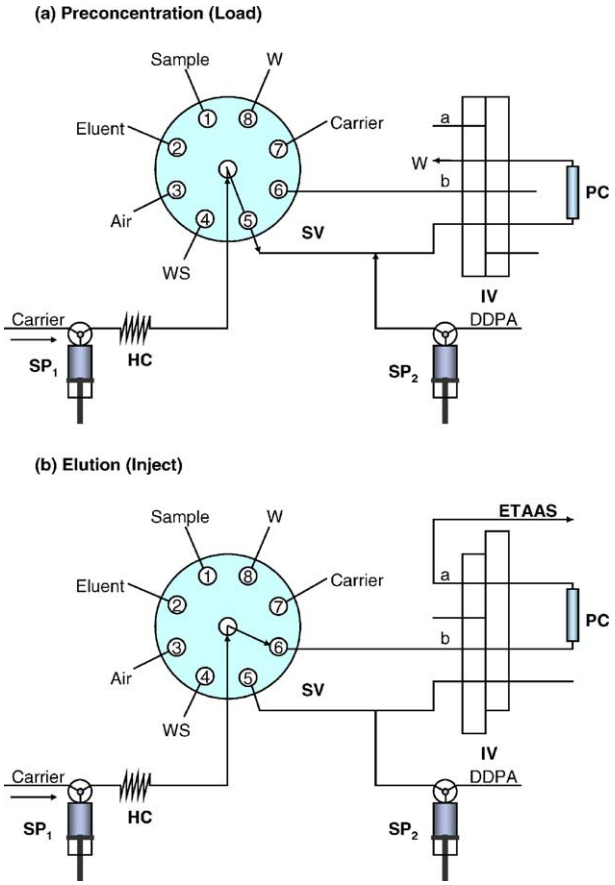

Fig. 1. Schematic diagram of the SI on-line column separation/preconcentration system with detection by ETAAS: (a) load position; (b) inject position. SP_1 and SP_2: syringe pumps; SV: 8-port selection valve; PC: packed column; HC: holding coil; IV: 2-position injection valve; eluent: ethanol; carrier solution: 0.05% HNO_3; WS: washing solution (0.2% (w/v) DDPA); W: waste; DDPA (0.8% w/v).

The SI-ETAAS system with external packed column (Fig. 1): A FIAlab-3000 system (FIAlab, Bellevue, WA, USA), equipped with two syringe pumps, SP_1 and SP_2, (Cavro, Sunnyvale, CA, USA) with capacities of 10 and 2.5 ml, respectively, was used. The SI system consisted of an external 8-port selection valve (SV) and a 2-position injection valve (IV) (VICI Valco Instruments Co. Inc. TX, USA). The holding coil (HC) was made by PTFE tubing (1.32 mm i.d./1.93 mm o.d.). All outlets of the SV and the IV were connected through PEEK ferrules with rigid PTFE tubing (0.5 mm i.d./1.60 mm o.d.), while the waste tube had the dimensions 1.0 mm i.d./1.6 mm o.d. The packed column, filled with 5 mg of PTFE beads, was made by a piece of PTFE tube (1.39 mm i.d./2.0 mm o.d.; active length ca. 4 mm) and blocked by glass wool at both ends.

The SI-LOV–ETAAS system (Fig. 2): The above-mentioned FIAlab-3000 system was used. The SI system consisted of a 6-port multiposition valve mounted with the integrated lab-on-valve microsystem [18], and an external peristaltic pump (Ismatec, Glattbrugg, Zurich, Switzerland) with Tygon pump tubing (0.95 mm i.d./2.67 mm o.d.). Both

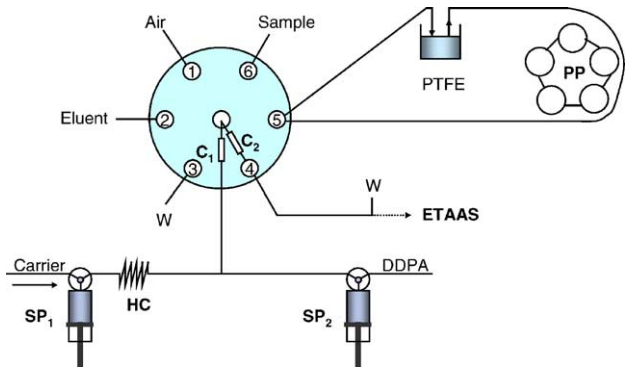

Fig. 2. Schematic diagram of the SI-LOV system for ETAAS detection of ultratrace levels of cadmium using PTFE beads. SP_1 and SP_2: syringe pumps; C_1 and C_2: microcolumns; PP: peristaltic pump; PTFE: poly(tetrafluoroethylene) bead suspension; eluent: mixture of ethanol and 0.5% Triton X-100; carrier: 0.05% HNO_3; W: waste; DDPA (0.8% w/v).

syringe pumps were connected to the valve through a PTFE mixing tee. The holding coil was made by PTFE tubing (1.32 mm i.d./1.93 mm o.d.). The LOV microsystem (material: PVC, diameter: 5 cm, thickness: 1 cm) contains six micro channels (1.66 mm i.d./12.0 mm length); every channel can communicate with a central port by a conduit in the selection valve. Two channels in the LOV (the central one and the one linked to port number 4) serve the roles of microcolumns (C_1 and C_2) for the PTFE beads, the beads being withheld by small PEEK stoppers, which will retain the beads, yet allow the solutions to flow freely. All channels were connected by PEEK ferrules and rigid PTFE tubing (0.8 mm i.d./1.60 mm o.d.).

The ETAAS instrument was synchronized with the FIAlab system, although the SI or SI-LOV systems were controlled by a computer, operated with FIAlab software, independent of that of the spectrometer.

2.2. Reagents

The PTFE granular Algoflon® S 131, type IV. G. 2 (Solvay Solexis S.p.A, Italy) beads (average size 650 μm) were sieved to obtain the fraction with sizes from 88 to 125 μm to facilitate the operation in the LOV system. Similarly, beads with the same size were selected as packing material for the external column. The packed column was filled with 5 mg PTFE Algoflon® beads, corresponding to the same amount of beads used in the LOV. The Aldrich PTFE beads have been described previously [18].

All the reagents were of at least analytical-reagent grade. Milli-Q water was used throughout. Working standard solutions of cadmium were prepared by diluting 1000 mg l^{-1} stock standard solution (Merck). 0.8% and 0.2% ammonium diethyldithiophosphate (DDPA, Aldrich) solutions were prepared by dissolving 0.8 g and 0.2 g of DDPA in 100 ml of water, respectively. Other chemicals were: Suprapur nitric acid (65%, Merck), Suprapur perchloride acid

(70%, Merck), fluoric acid (40%, Merck), and absolute ethanol.

2.3. Sample pretreatment

Three standard reference materials were chosen: Community Bureau of Reference CRM 279 (Sea Lettuce), Community Bureau of Reference CRM 320 (River Sediment), and SRM 1640 (Natural Water) standard from the National Institute of Standard and Technology (NIST).

After weighing 0.2 g of CRM 279 (or 0.5 g of CRM 320), 3.0 ml of nitric acid (65%), and 3.0 ml of fluoric acid (40%) were added to the PTFE vessel used, and the solution was heated gently to near dryness in a sand bath, care being taken that the temperature did not exceed 140 °C. After that the samples were cooled and 1 ml of perchloric acid was added. The samples were then heated again to near dryness. Finally, the samples were diluted to 100 ml with 2% of nitric acid. Three milliliters of the SRM 1640 was diluted directly to 100 ml by 2% of nitric acid. All the sample solutions were then further properly diluted to make the analyte concentrations within the linear dynamic range of the instrumental procedure.

2.4. Operating procedure

Tables 1 and 2 list the individual steps for the SI system with external packed column and the SI-LOV system with renewable microcolumn, respectively. More explicitly, Table 1 summarises the basic features of the SI-analytical procedure, comprising the analyte derivatization/sorption manipulations (steps 1–8), elution and transportation of the eluent (steps 9–12), and finally cleaning (steps 14–17).

By the same token, Table 2 summarises the individual steps of the SI-LOV analytical procedure, comprising precondition (steps 1–3), bead loading and derivatization/sorption (steps 4–6), cleaning (step 7), elution and eluate transportation (steps 8–12), and beads discarding (steps 14–17).

3. Results and discussion

3.1. Optimization of ETAAS parameters

Firstly, the effects of the pyrolysis and atomization temperatures on the determination of cadmium were investigated. The optimum conditions are shown in Table 3. It was observed that significant loss of analyte took place at pyrolysis temperatures higher than 350 °C, leading to drastically reduced signals. The optimal atomization temperature was found to be 1400 °C, resulting in the highest analytical signals and also well-shaped peaks. With reference to the information previously reported [9], a pyrolysis temperature of 350 °C, a holding time of 40 s, along with an atomization temperature of 1400 °C were finally selected.

3.2. Optimization of chemical variables

The effects of the concentration of DDPA and the sample acidity have been investigated thoroughly earlier [9], and therefore a DDPA concentration of 0.8% (w/v) and a nitric acid concentration of 2.0% (v/v) were adopted.

3.3. Optimization of SI parameters

The effect of sample flow rate and loading time were both investigated by fixing the DDPA flow rate at 12 $\mu l\,s^{-1}$ and the sample volume at 1250 μl and changing the sample loading flow rate to obtain a sample:DDPA flow rate ratio within a range from 1 to 3. As it turned out, the observed variations in the integrated absorbance as a function of the sample flow rate were rather limited below a ratio of 2, while at sample flow rates higher than 30 $\mu l\,s^{-1}$ a decrease was recorded. A sample flow rate of 24 $\mu l\,s^{-1}$ was thus employed for further investigation.

By fixing the sample flow rate:DDPA flow rate ratio at 2, the integrated absorbance increased with increasing sample loading time. No analyte breakthrough was observed at sample volumes up to 2.0 ml (0.1 $\mu g\,l^{-1}$ of Cd). In the present study, a sample loading time corresponding to a sample volume of 1250 μl was used.

In order to remove the remaining non-adsorbed or weakly adsorbed constituents of the matrix in the packed column after preconcentration a cleaning step was found necessary before initiating the elution step. A washing solution comprising a suitable amount of chelating reagent is recommended to prevent loss of analyte. Experiments showed that even water and diluted nitric acid solution, which are commonly used as washing solution in SI system, could cause significant loss of analyte. Therefore, a 0.2% DDPA solution was used as washing solution.

Since absolute ethanol has been found to be most effective for elution of the adsorbed complex [9], it was also used herein. A volume of 50 μl of eluent sufficed to obtain quantitative elution of the analyte adsorbed on the packed column. Smaller volumes gave rise to non-complete elution and caused reduced signals, while larger volumes did not result in noticeable increase of signals. Considering the limited capacity of the graphite tube, a volume of 50 μl eluent was finally selected. The flow rate of the eluent had merely marginal effect on the recorded signals when it was below 9 $\mu l\,s^{-1}$, while the signals became reduced when the flow rate was higher than 9 $\mu l\,s^{-1}$. So, an eluent flow rate of 7 $\mu l\,s^{-1}$ was employed.

3.4. Investigating the feasibility of exploiting the new PTFE beads in the renewable surface microcolumn LOV system

As stated above, two of the most essential requirements of a sorbent material to be satisfied when a renewable fashion is to be pursued are the bead size homogeneity and a spherical

Table 1
Protocol for automated cadmium determination by the SI system with external packed column interfaced with ETAAS detection

Step	SP_1	SP_2	lSV position	lIV position	Command	Commentary
1	Out	In	1	1	Aspirate 1250 μl of sample at 100 μl s^{-1} Aspirate 625 μl of DDPA at 50 μl s^{-1}	Sample and chelating reagent loading
2	Out	Out	5	1	Dispense 1250 μl of sample at 24 μl s^{-1} Dispense 625 μl of DDPA at 12 μl s^{-1}	Analyte derivatization and sorption
3	Out	Out	3	1	Aspirate 400 μl of air at 100 μl s^{-1}	Aspirate air to HC
4	Out	Out	4	1	Aspirate 240 μl of washing solution at 80 μl s^{-1}	Aspirate washing solution to HC
5	Out	Out	5	2	Dispense 640 μl of washing solution and air at 24 μl s^{-1}	Rinsing column
6	Out	Out	3	2	Aspirate 800 μl of air at 100 μl s^{-1}	Aspirate air to HC
7	Out	Out	6	2	Dispense 800 μl of air at 60 μl s^{-1}	Fill ETAAS line with air
8	Out	Out	3	2	Aspirate 1000 μl of air at 100 μl s^{-1}	Aspirate air to HC
9	Out	Out	2	2	Aspirate 50 μl of eluent at 10 μl s^{-1}	Eluent loading
10	–	–	–	–	Activation of ETAAS program	
11	Out	Out	6	2	Dispense 50 μl ethanol plus 800 μl air at 7 μl s^{-1}	Elution and eluate transportation via air segmentation to ETAAS
12	Out	Out	6	2	Dispense 200 μl of air at 30 μl s^{-1}	
13	–	–	–	–	Running ETAAS program	Autosampler tip out from the furnace
14	Out	Out	3	2	Aspirate 400 μl of air at 100 μl s^{-1}	Aspirating air
15	Out	Out	7	2	Aspirate 400 μl of carrier solution at 100 μl s^{-1}	Carrier loading
16	Out	Out	2	2	Aspirate 400 μl of ethanol at 100 μl s^{-1}	Ethanol loading
17	Out	Out	6	2	Empty all the air, carrier and ethanol in HC at 60 μl s^{-1}	Cleaning column

SP_1: syringe pump no. 1; SP_2: syringe pump no. 2; SV: selection valve; IV: injection valve; HC: holding coil.

shape of all entities in order to prevent a compact settlement in the microcolumn positions. When observed under a microscope, the Aldrich PTFE beads appeared more like lumps of irregular shape and were characterized by very non-uniform size, and were relatively difficult to handle within the LOV conduits. The new granular PTFE beads, on the other hand, have a much better morphology, proving to be almost round-shaped. Therefore, this kind of beads were to be expected to show a better physical performance when exploited in the SI-LOV system. The operational sequence of the SI-LOV system with renewable microcolumn is described above (Section 2.4, Table 2).

Due to the hydrophobic nature and the high density of the PTFE beads, organic solvents and a stirred reservoir are indispensable to form a stable and homogeneous PTFE bead suspension for reproducible manipulation within the LOV system (re Fig. 2). Various organic reagents, such as non-ionic surfactant Triton X-100, ethanol, butanol, glycol, and mixtures of Triton X-100 and ethanol of different concentrations were evaluated. The experimental results showed that absolute ethanol with Triton X-100 (0.5% v/v) exhibited the best performance in the circulation and aspiration of the PTFE bead into the LOV microcolumn.

Table 2
Protocol for automated cadmium determination by the SI-LOV system with renewable microcolumn interfaced with ETAAS detection

Step	SP_1	SP_2	PP	LOV position	Command	Commentary
1	In	In	On	–	Aspirate 800 μl of carrier solution at 100 μl s^{-1} Aspirate 625 μl of DDPA at 100 μl s^{-1}	System precondition
2	Out	In	On	2	Aspirate 300 μl of ethanol at 20 μl s^{-1}	
3	Out	In	On	4	Dispense 500 μl (200 μl ethanol and 300 μl carrier solution) at 30 μl s^{-1}	
4	Out	In	On	6	Aspirate 1250 μl sample at 100 μl s^{-1}	Sample loading
5	Out	In	On	5	Aspirate 60 μl of bead suspension at 4 μl s^{-1}	Beads loading in column C_1
6	Out	Out	Off	4	Dispense 1250 μl of sample at 24 μl s^{-1} Dispense 625 μl of DDPA at 12 μl s^{-1}	Analyte derivatization and sorption in column C_2
7	Out	Out	Off	4	Dispense 300 μl of carrier solution at 24 μl s^{-1}	Bead rinsing
8	Out	Out	Off	1	Aspirate 580 μl of air at 40 μl s^{-1}	Fill ETAAS line with air
9	Out	Out	Off	4	Dispense 330 μl of air at 30 μl s^{-1}	
10	–	–	–	–	Activation of ETAAS program	Autosampler tip into the furnace
11	Out	Out	Off	2	Aspirate 50 μl of ethanol at 10 μl s^{-1}	Eluent loading
12	Out	Out	Off	4	Dispense 50 μl ethanol plus 250 μl air at 7 μl s^{-1}	Elution and eluate transportation via air segmentation to ETAAS
13	–	–	–	–	Running ETAAS program	Autosampler tip out from the furnace
14	Out	Out	Off	2	Aspirate 300 μl of ethanol at 100 μl s^{-1}	Discarding used beads to waste (steps 14–17)
15	Out	Out	Off	4	Dispense 200 μl of ethanol at 50 μl s^{-1}	
16	Out	Out	Off	4	Aspirate 188 μl of beads and ethanol at 100 μl s^{-1}	
17	Out	Out	Off	3	Empty beads, ethanol and carrier at 100 μl s^{-1}	

SP_1: syringe pump no. 1; SP_2: syringe pump no. 2; PP: peristaltic pump; HC: holding coil.

Some physical precautions were taken to improve the performance of transporting, aspirating, and discarding the PTFE bead: thus, a rather large-bore transport line (1.00 mm i.d.) and peristaltic pump tubing with the same internal diameter were used for beads circulation. All the connecting parts in the circulating system were ensured to possess similar internal diameter, and all the connection conduits between the LOV and the selection valve grooves were enlarged from 0.5 to 1.5 mm. By these measures, and by suitable choice of organic solvent, a better performance of manipulating the suspended bead into and within the LOV microsystem was obtained. The preliminary experimental results showed that the granular PTFE beads exhibited higher sensitivity when compared with the Aldrich beads when operated in the renewable fashion.

However, the morphology of the granular PTFE bead underwent change in the long run, the beads breaking into smaller particles, which tended to pack and get adhered both within the circulating system and the LOV microcolumns. This can very likely be ascribed to a "memory effect" because the commercial Algoflon® beads, which are intended for coating purposes, are actually produced on the basis of small particles (25 μm), which through a manufacturing process are made into a product of different beads sizes with a nominal diameter of 650 μm. When subjected to mechanical stimulus, they are obviously broken down to their original entities, which in the environment of the organic solvent induces them to aggregate. This was actually verified by suspending the bead material in a high concentration of non-ionic surfactant (Triton X-100), where it was observed that the

Table 3
Operating parameters of the graphite furnace program

Step	Temperature (°C)	Ramp time (s)	Holding time (s)	Argon flow rate (ml min^{-1})
Preheating	90	5	10	250
Drying	160	5	30	250
Pyrolysis	350	15	35	250
Atomization	1400	0	5	0
Cleaning	2600	1	2	250

suspension soon turned into an emulsion. Besides, the higher hydrophobicity of the small Algoflon® beads, as compared to the previously used PTFE bead, leads them to pack together more readily. So difficulties in circulating, aspirating and discarding the granular PTFE bead were not infrequently encountered.

Moreover, the introduction of the surfactant led to the presence of a high background signal, the source of which currently is under investigation. In order to evaluate the analytical characteristics and performance of the granular PTFE beads it was, therefore, decided to examine this material in a sequential injection system furnished with permanent packed column, and then evaluate the results in comparison with those obtained earlier with the Aldrich PTFE beads in an SI-LOV system.

3.5. Analytical performance of the granular PTFE beads in an SI system with a permanent packed column

To evaluate the analytical performance of the granular PTFE beads and the Aldrich ones under the comparable conditions, a small packed column, which imitates the amount of beads in the SI-LOV system, was employed. Thus, 5 mg of the granular PTFE bead was used in the packed column, which is equal to the beads of the 60 μl of PTFE bead suspension used in the LOV microcolumn. The operational sequence of the SI-external packed column system is described above (Section 2.4, Table 1).

The analytical figures of merits of the packed column in the SI system using the granular PTFE bead as compared to the renewable microcolumn in the LOV system with Aldrich PTFE beads are listed in Table 4, including statistical parameters, sample throughput, sample, reagent and beads consumption, retention efficiency and enrichment factor. The enrichment factor is calculated by comparison of the integrated absorbance of the target analyte after the SI pretreatment with that recorded by direct injection of 50 μl of a standard solution in 2% nitric acid (v/v). The retention efficiency was calculated by comparison of the analytical signal obtained following chelate sorption with that measured by direct injection of the total amount of loaded analyte, assuming quantitative elution of the loaded beads. The repeatability was expressed as the precision obtained by six consecutive injections of a 0.1 μg l^{-1} cadmium standard solution using a permanent column, whereas the reproducibility was given as the coefficient

Table 4
Analytical performance of the external packed column using the granular PTFE bead in the SI system as compared to the renewable microcolumn with Aldrich PTFE bead in the LOV system for the determination of Cd by ETAAS

Parameter	Granular PTFE bead	Aldrich PTFE bead
Regression equation (n = 5)	AA = 1.6107[Cd] (μg l^{-1}) + 0.0040	AA = 0.2956[Cd] (μg l^{-1}) + 0.0017
Linear calibration range (μg l^{-1})	0.05–0.25	0.05–1.00
Correlation coefficient	r = 0.9991	r = 0.9988
Detection limit (ng l^{-1}) ($3\sigma_{blank}$, n = 6)	5.5	5.0
Repeatability (%) (0.1 μg l^{-1}, n = 6)	1.3	3.0
Reproducibility (%) (0.1 μg l^{-1}, n = 4)	1.0	4.3
Bead consumption	5 mg (equal to 60 μl)	60 μl
Sample volume (μl)	1250	1250
Sample frequency (h^{-1})	12	12
Sample loading flow rate (ml min^{-1})	1.44	1.44
Reagent loading flow rate (ml min^{-1})	0.72	0.72
Retention efficiency (%)	82	74
Enrichment factor	20.6	17.2

of variation obtained with four different columns using the same standard solution.

As seen, the granular PTFE bead exhibited much improved sensitivity, that is, from 0.2956 to 1.6107 in comparison with the Aldrich beads. The packed column yielded much better repeatability and reproducibility values. The reason is that it used the same batch of beads for a series of analytical runs, while in the LOV the amount of beads was controlled by the volume of the beads suspension and the amount of beads aspirated in each analytical run, which might not be perfectly reproducible from run to run because of the non-homogeneity of the bead suspension. The enrichment factor and retention efficiency were improved from 17.2 to 20.6 and from 74 to 82%, respectively. The detection limit of the proposed method was similar to that found earlier, but this one is very likely dictated by the purity of the reagents used. The linearity range of the method (0.05–0.25 μg l^{-1}) was somewhat smaller than that obtained before. However, that is not too surprising considering the high sensitivity of the signal.

In the packed column any difficulties of transportation and discarding beads are avoided and the beads can be used repeatedly and disposed intermittently, while the major drawback of the PTFE lumps in the LOV system is the inherent ability of tighter packing of the beads in the microconduits.

When columns of larger volumes were prepared, clogging problems started to arise at high flow rates or over extended

Table 5
Determination of cadmium in certified reference materials

Sample	Certified value ($\mu g\,g^{-1}$)	Found value ($n = 4$) ($\mu g\,g^{-1}$)	Recovery (%)
CRM279	0.274 ± 0.022	0.273 ± 0.013	99
CRM320	0.533 ± 0.026	0.516 ± 0.006	96
SRM1640	22.79 ± 0.96	22.30 ± 1.13	97

time of use if more than 75 mg of beads were packed. However, when using the small columns with 5 mg of beads the function was entirely trouble-free: neither backpressure effects nor any tendency of packing was observed even after long-term uni-directional operation. Besides, eluting the column completely with a well-defined, small volume of eluate was readily feasible.

Therefore, from an analytical point of view the improvements in the operational characteristics reveal the granular PTFE bead to constitute a more potent and promising material for implementing separation and preconcentration than the Aldrich PTFE beads. However, since the new PTFE material does not possess sufficient mechanical stability, its use in the LOV scheme is, at this point, restricted, and it is to be hoped that the material can be obtained with sufficiently improved physical qualities.

3.6. Investigation of interferences

It has been reported that DDPA forms stable chelates merely with a few transition and heavy metals including Ni(II), Cu(II), Co(II), Fe(III), and Pb(II) [1,19]. In order to evaluate potential interferents on the target species, both in the complexation reaction and in the chelate sorption process onto the surface of the granular PTFE packed column, different concentrations of the mentioned metals were added to $0.1\,\mu g\,l^{-1}$ solutions of cadmium and then analyzed.

Maximum interferent:analyte ratios of 10^5 for Ni(II) and Fe(III), 10^4 for Co(II), and 10^3 for Cu(II) were readily tolerated. Pb(II) was the most severe interferent in the system, allowing only a maximum ratio of 100. However, by masking the Pb(II) by a suitable reagent, such as thiocarbamide, substantially higher tolerance levels to this metal are readily possible [18].

3.7. Applications of the SI system with external packed column

In order to examine the effectiveness of the granular PTFE packed column approach, the system was applied to the determination of trace levels of cadmium in three certified reference materials: CRM 279 (Sea Lettuce), CRM 320 (River Sediment), and SRM 1640 (Natural Water). The experimental results showed that the concentrations of cadmium determined were in good agreement with the certified reference values, with percentage recoveries of 99, 96, and 97 for CRM 279, CRM 320, and SRM 1640, respectively (Table 5).

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Paper II

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Determination of trace metal ions via on-line separation and preconcentration by means of chelating Sepharose beads in a sequential injection lab-on-valve (SI-LOV) system coupled to electrothermal atomic absorption spectrometric detection

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Abstract

The analytical performance of an on-line sequential injection lab-on-valve (SI-LOV) system using chelating Sepharose beads as sorbent material for the determination of ultra-trace levels of Cd(II), Pb(II) and Ni(II) by electrothermal atomic absorption spectrometry (ETAAS) is described and discussed. The samples are adjusted to pH 5.0 on-line in the system for optimum operation. The target ions are adsorbed by chelation on the surface of the beads, contained in a 20 μl microcolumn within the LOV, and following elution by 50 μl 2 M nitric acid, the eluate is, as sandwiched by air segments, introduced into the ETAAS. Based on the consumption of 1.8 ml sample solution, retention efficiencies of 95, 75 and 90%, enrichment factors of 34, 27 and 32, and determination limits of 0.001, 0.07 and 0.02 $\mu g\,l^{-1}$ were obtained for Cd(II), Pb(II) and Ni(II), respectively. The beads can be used repeatedly for at least 20 times without decrease of performance, yet can be replaced at will if the circumstances should so dictate. The optimized procedural parameters showed that 12 samples per hour could be prepared and successfully analyzed. The results obtained for three standard reference materials agreed very well with the certified values.

Keywords: On-line separation and preconcentration; Sequential injection lab-on-valve; Electrothermal atomic absorption spectrometry; Chelating Sepharose; Cd; Pb; Ni

1. Introduction

The determination of metals at trace and ultra-trace levels in complex matrices, such as biological and environmental samples, still pose as one of the challenging areas in analytical chemistry [1]. Although ETAAS is one of the most sensitive and matured techniques for the determination of these constituents, the extremely low concentrations encountered, often in the presence of complex matrix interfering constituents, make their direct analysis difficult. As a consequence, sample pretreatment with preconcentration and separation from the interfering matrix prior to measurement by ETAAS is frequently required.

Various separation and preconcentration schemes based on batch or flow injection (FI) modes have been developed, including solvent extraction [2,3], solid-phase extraction [4,5], precipitation [6,7], or hydride and vapor generation [8,9]. Seen from an on-line operational point of view, the methods based on sorbent extraction have proven to be the most attractive ones, and have thus been extensively studied, not the least because of their high separation and preconcentration efficiency, but also because they can readily be implemented and controlled. In this context, micro-particles/beads are often employed as the solid phase for analyte extraction. Beads with different size, of various materials, and with

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diverse functional groups, are nowadays commercially available. Furthermore, their functional groups can be modified for special applications.

Reported sorbent materials include chelating ion exchangers such as Chelex-100, immobilized 8-hydroxyquinoline and dithizone-modified Sephadex G-25 [10]; anion and cation exchangers such as Sephadex C-25 [11,12]; activated carbon [13]; C_{18}-silicagel [14,15]; octadecyl-chemically modified poly(styrenedivinylbenzene) copolymers (C_{18}-PS/DVB) [16]; poly(tetrafluoroethylene) (PTFE) [17–19]; or simply knotted reactors (KR) [20–22].

Amongst the chelating ion-exchangers, the most common functional group used is iminodiacetic acid (IDA). This sorbent strongly binds transition metal ions through the interaction between the iminodiacetic groups and the electron-free d-orbits of the metal elements. However, IDA-containing sorbents based on hydrophobic organic polymers show considerable volume changes in different media and/or low sorption rates [23]. Therefore, the introduction of a more hydrophilic support is of great interest. A highly cross-linked agarose with IDA functional groups, called Novarose, has been studied as adsorbent for metal ions [24–26], its transferring rate for the preconcentration of transition elements in the batch mode having been reported to be 50 times faster than that for Chelex-100 [24].

A potentially very promising candidate for use in the SI-LOV mode is the chelating Sepharose Fast Flow material (Amersham Biosciences, Sweden), consisting of iminodiacetic acid groups coupled to Sepharose 6 Fast Flow by stable ether linkages and sufficiently long spacer arms. The support part is a highly cross-linked and hydrophilic 6% agarose with excellent chemical and physical stability. The rigid base matrix permits very high flow velocities. Originally intended to be used for immobilized metal ion affinity chromatography [27], the size of the commercially available beads therefore ranges from 45 to 165 μm. Such size is perfectly suited for use and manipulations in on-line systems, not the least because the beads exhibit negligible volume variations due to changes in pH or ionic strength. Besides, the material is chemically stable under both acidic and alkaline conditions, and under the microscope the beads appear of perfectly spherical shape.

Conventionally, sorbent extraction schemes make use of an external stationary column [28,29] operated in a permanent fashion, which often leads to problems such as progressively tighter packing of the sorbent material, increase of flow resistance [30] and irreversible changes of its surface properties. Bi-directional flows during sample loading and eluting [23], or intermittent back aspiration of small air segments [30], can partly solve these problems. Yet, to overcome all the shortcomings, the concept of the renewable surface for each individual assay, that is the sequential injection lab-on-valve (SI-LOV) approach [31], is preferentially used.

SI-LOV is the so-called third generation of FIA [32,33]. The LOV itself is a microconduit, with 6, 8 or 10 external ports, made from hard PVC and mounted atop of a multiposition valve. Containing a channel array permitting various unit operations to be implemented on-line, the LOV is communicating with a high precision syringe pump via a holding coil, wherein liquid zones (sample and reagent(s)), aspirated from the individual ports, initially are stacked one after the other, and later forwarded to allow chemical reaction(s) and/or physical operations (such as retention of species on an incorporated column) to take place, followed by detection of the analyte. All operations are controlled by a computer and can be appropriately programmed. For channels used as microcolumn positions, small pieces of PEEK tubing act as stoppers at each outlet to trap the beads, yet to allow solutions to flow freely [33].

In this work the performance of the potentially very promising chelating Sepharose beads are applied in the SI-LOV mode for the determination of ultra-trace amounts of Cd, Pb and Ni in biological and environmental samples. Cd and Pb are highly toxic elements and their concentration in environmental samples and body fluids and tissues are of main concern in the studies of environmental pollution and occupational exposure [34,35]. Ni is an essential element for human heath, but some of its compounds are carcinogenic [36].

The accuracy of the method suggested was corroborated by the analysis of biological reference materials, that is, CRM 320 (River sediment) and BCR No. 279 (Sea lettuce) from The Community Bureau of Reference (BCR) and SRM 1640 (Natural water) from The National Institute of Standards and Technology (NIST).

2. Experimental

2.1. Instrumentation

A diagram of the SI-LOV-ETAAS system used is schematically shown in Fig. 1. A Zeeman atomic absorption spectrometer (Perkin-Elmer AAnalyst 600) equipped with a Transversely Heated Graphite Atomizer (THGA) furnace was employed. The Cd hollow cathode lamp (S&J Juniper & Co., England) was used at a current of 5 mA and at wavelength/spectral bandpass of 228.8/0.7 nm. The Pb hollow cathode lamp (S&J Juniper & Co., England) was operated at a current of 10 mA and at wavelength/spectral bandpass of 283.3/0.7 nm. The operating condition for the Ni hollow cathode lamp (Perkin-Elmer) was a current of 25 mA and at wavelength/spectral bandpass of 232.0/0.2 nm. Integrated peak area mode was used for recording the results in all cases.

A FIAlab-3000 system (FIAlab, Bellevue, USA), equipped with two syringe pumps (SP1, volumetric capacity 10 ml; and SP2, 2.5 ml) and a peristaltic pump (PP), was used. It included a 6-ports selection valve (SV) mounted with the integrated LOV microsystem [12]. The LOV, made from PVC, contains six microchannels (1.66 mm i.d./12.0 mm length), the peripheral ports of which (1–6) can be made to address the central port of the LOV via the central communication channel in the SV. One of the outlets is split into two ports

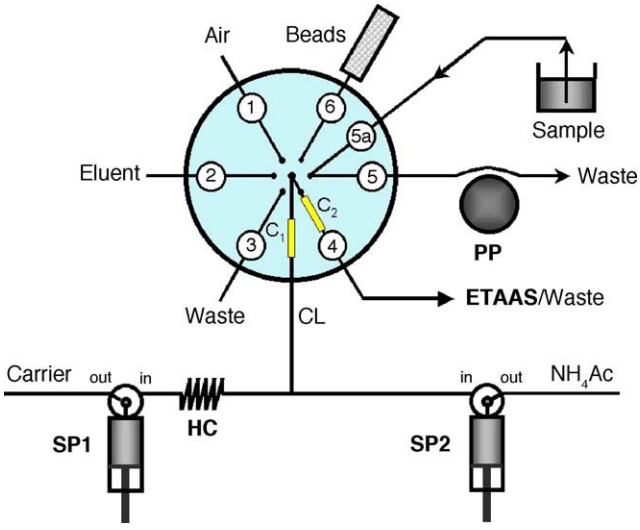

Fig. 1. SI-LOV system for determination of ultra-trace levels of Cd, Pb and Ni using preconcentration by chelating Sepharose beads and detection by ETAAS. SP1 and SP2, syringe pumps; PP, peristaltic pump; CL, communication line; C_1 and C_2, microcolumn positions (although these for clarity are shown wider, all channels in the LOV are actually of identical internal diameter (1.66 mm)).

(here 5/5a). External connection from the LOV to the syringe pump(s) is made from the central port via the communication line (CL). Two of the channels in the LOV act as microcolumn positions (C_1 (within CL) and C_2) for trapping the beads by means of small PEEK stoppers at the ends. The holding coil was made from PTFE tubing (1.32 mm i.d./1.93 mm o.d.; length 185 cm, corresponding to a volume of 2.5 ml). All the other connecting tubings were made from rigid PTFE (0.60 mm i.d./1.60 mm o.d.). The peristaltic pump, which was furnished with a Tygon pump tube (1.22 mm i.d./2.80 mm o.d., allowing for a flow rate of 2.1 ml min^{-1}), was via port 5 connected to port 5a via their common outlet at the center of the LOV and hence to the outer sample reservoir. Thereby, a virtually zero dead sample volume was ensured, effectively preventing carry-over from sample to sample.

The ETAAS instrument and the FIAlab system were controlled by two different, independent computers, the operations of which were, however, synchronized.

pH measurements were effected by a digital pH meter (PHM92 LABpH Meter, Radiometer Danmark A/S).

2.2. Reagents

Commercially available Chelating Sepharose^{TM} Fast Flow beads (Amersham Biosciences) were received from the manufacturer in 20% ethanol solution. This suspension was used directly in the syringe mounted as bead reservoir in port 6 of the LOV [12].

All the reagents used were of analytical-reagent grade. All the series of cadmium, lead and nickel standard solutions were prepared by appropriate dilution of 1000 mg l^{-1} stock standard solutions (Merck) with 0.1 M HNO_3. The carrier stream was a 0.01 M acetate buffer adjusted to pH 5.0. A 1 M ammonium acetate solution, prepared by dissolving 15.4 g of the salt in 200 ml of water (pH 6.6), was used as on-line pH adjustment agent. Other reagents used were Suprapur nitric acid (65%, Merck), Suprapur perchloric acid (70%, Merck) and hydrofluoric acid (40%, Merck).

2.3. Sample pretreatment

The reference materials used were CRM 279 (Sea Lettuce) and CRM 320 (River Sediment) from The Community Bureau of Reference, and SRM 1640 (Natural Water) from The National Institute of Standard and Technology (NIST). The first two materials were pretreated as follows: 0.5 g of CRM 279 (or CRM 320) was weighted and placed into PTFE vessels. To each vessel was added 6.0 ml of nitric acid (65%) and 3.0 ml of hydrofluoric acid (40%). The samples were then heated gently to near dryness in a sand bath, the temperature not exceeding 140 °C. The solutions were cooled and 1 ml of perchloric acid was added, whereafter the samples were again heated to near dryness. Finally, 2.0 ml of 65% nitric acid was added to the residue and the solution was transferred to a 100 ml volumetric flask and diluted to the mark with deionized water. The liquid SRM 1640 was diluted by 0.1 M nitric acid directly. The individual sample solutions were then further appropriately diluted by 0.1 M nitric acid to make the analyte concentrations within the linear dynamic range (Table 4).

2.4. Operating procedure

The detailed operating procedure of the SI-LOV system is listed in Table 1. The main four functional sequences are summarized as follows:

- *Column pretreatment/cleansing* (steps 1–8). Syringe pump SP1 is set to aspirate 700 μl of carrier solution (0.01 M acetate of pH 5.0) from the external reservoir, and then, sequentially, into the holding coil (HC), 400 μl of 2 M nitric acid from the eluent port 2 and 20 μl chelating Sepharose beads suspension (port 6). At the same time, syringe pump SP2 is set to aspirate 450 μl of the 1 M ammonium acetate agent for pH adjustment. The beads are withheld in the LOV forming microcolumn C_1. Then 400 μl of 2 M nitric acid followed by 300 μl of the carrier are dispensed through port 4, whereby the beads are cleansed and washed to pH 5.0. During this operation, the beads are transported from column position C_1 to form the microcolumn C_2 in the channel corresponding with port 4.
- *Analytes loading* (steps 9–12). A sample volume of 1800 μl is aspirated from port 5a by SP1. Then this volume is dispensed and mixed with the pH adjusting solution from SP2. The mixed solution passes through column C_2, where the target ions are chelated to the beads, while the matrix solution via port 4 goes to the waste.

Table 1
Operational sequences of the SI-LOV system for the determination of Cd, Pb and Ni by ETAAS

Step	SP1	SP2	LOV position	Action	Flow rate (μl s^{-1})	Volume (μl)
1	Out	–	–	Aspirate carrier	100	700
2	–	Out	–	Aspirate NH_4Ac	100	450
3	In	–	2	Aspirate eluent	50	400
4	In	–	6	Aspirate beads	5	20
5	In	–	4	Dispense eluent	10	100
6	–	–	–	Delay 5 s		
7	In	–	4	Dispense eluent	20	300
8	In	–	4	Dispense carrier	20	300
9	In	–	5	Aspirate sample	100	1800
10	In	–	4	Dispense sample	40	1800
11	–	In	4	Dispense NH_4Ac synchronically with step 10	10	450
12	In	–	4	Dispense carrier	50	150
13	In	–	1	Aspirate air	50	580
14	In	–	4	Dispense air	20	380
15	–	–	–	Activation of ETAAS		
16	In	–	2	Aspirate eluent	10	50
17	In	–	4	Dispense eluent	10	50
18	–	–	–	Delay 5 s		
19	In	–	4	Dispense air	10	200
20	In	–	4	Dispense carrier	10	200
21	–	–	–	Run ETAAS program		
22	In	–	4	Aspirate carrier	50	200
23	In	–	3	Discard beads and carrier	50	270

"Out" means that the pertinent syringe pump communicates with an external reservoir/solution; "in" means that it communicates with the SI-LOV system.

Afterwards, the sample is replaced by the next one, and the sample lines via port 5a and 5 are filled with fresh sample solution by activating the peristaltic pump (PP).

- *Elution* (steps 13–21). SP1 is set to aspirate 580 μl of air from port 1 and then dispense 380 μl of the air via C_2 to fill the ETAAS line. Then 50 μl of eluent is aspirated from port 2, and at the same time the ETAAS is activated. After that, the eluent is dispensed to the analyte-loaded beads and is stopped there, remaining for a period of 5 s, whereupon all the eluate is transported to the graphite tube by the remaining air (200 μl) plus 200 μl of carrier solution, that is, the eluate is sandwiched during the transport by air segments to minimize dispersion. The ETAAS instrument runs the program and determines the analyte element.
- *Beads discarding* (steps 22 and 23). SP1 is set to aspirate the 200 μl of carrier remaining in the ETAAS line together with the beads from port 4, and then via port 3 discard them to the waste.

If the beads are to be reused for one or more sample cycles, this last sequence is eliminated. Under any circumstances, the beads will be pretreated and cleansed before the next analysis cycle (steps 1–8).

3. Results and discussion

3.1. Optimization of ETAAS parameters

The effects of the pyrolysis and the atomization temperatures and the holding time on the determination of Cd, Pb and Ni were investigated, albeit with due reference to the values recommended in the literature. The optimum conditions are shown in Table 2.

When the pyrolysis temperature for Cd exceeded 400 °C the signal started to decrease, because of loss of Cd due to volatilisation, so 350 °C was chosen. For Pb the analytical signal began to diminish when the pyrolysis temperature was higher than 500 °C. So 400 °C was selected for that element. For Ni, the analytical and the background signals were unaffected by pyrolysis temperatures in the range 900–1300 °C and of holding times from 10 to 50 s. It was observed, however, that the analytical signal became higher and more stable when using an atomization temperature around 2100 °C. So the finally adopted instrumental parameters were 1100 °C

Table 2
Graphite furnace programs for determination of Cd, Pb and Ni

Step	Temperature (°C)	Ramp time (s)	Holding time (s)	Argon flow rate (ml min^{-1})
Preheating	110	5	20	250
Drying	140	5	30	250
Pyrolysis				
Cd	350	10	30	250
Pb	400	10	30	250
Ni	1100	10	30	250
Atomization				
Cd	1400	0	2	0
Pb	1600	0	3	0
Ni	2150	0	5	0
Cleansing				
Cd	2400	1	3	250
Pb	2450	1	3	250
Ni	2500	1	4	250

and a holding time of 30 s for pyrolysis, and 2150 °C for the atomization temperature.

3.2. Optimization of sample acidity

The sample acidity is a key factor in the process of chelation of metal ions. At low pH the metal ions will not be adsorbed onto the surface, while at high pH values the metal will form hydroxides, so no free metal ions are at hand in the solution. Experiments showed that at pH around 5.0 was optimal for the three metal ions.

There are potentially two approaches to adjust the sample pH: one is to pre-buffer the samples to pH 5.0; another way is to adjust the pH on-line by an appropriate solution, such as 1 M ammonium acetate. The latter approach was used in this study, since all samples initially were prepared in acid solutions (0.1 M HNO_3). As seen in Fig. 1, this was affected by the use of a second external syringe pump, which was employed to accommodate the pH-adjustment agent, and which was filled with the solution prior to start of the assays. Then the sample and ammonium acetate solutions were dispensed simultaneously and passed through the microcolumn, thereby ensuring that the desired pH was attained in the mixture.

3.3. Optimization of SI-LOV parameters

3.3.1. Column cleansing

Column cleansing was found to be necessary to prevent a high blank. Although the information sheet for the Sepharose beads claims that they are metal free, a very high blank was observed when aspirating fresh portions of beads without prior cleansing. After cleansing with 2 M nitric acid the blank decreased to a very low level. The volume needed of the cleansing solution was studied, and it was established that 400 μl sufficed for the column pretreatment. Even at the beginning of each analysis cycle, when used beads were employed repeatedly, column cleansing was necessary. The reason is very likely that the eluting procedure does not elute the analyte completely with the 50 μl eluent solution used (Section 3.3.3).

3.3.2. Effects of sample flow rate

The effect of sample flow rate was investigated by fixing the sample volume at 1800 μl and changing the sample flow rate. The results showed that the observed variation in the integrated absorbance of Cd, Pb and Ni as a function of sample flow rate between 5 and 100 $\mu l\,s^{-1}$ were rather limited. Metal ions were quickly adsorbed on the surface of the beads. It has been reported that the Sepharose beads act 50 times faster than the Chelex-100 ones, and this can be explained by the difference in the hydrophobicities and the anchoring of the chelating group to the support [24]. Sorbents based on a hydrophilic support appear to be faster than those based on an organic polymer matrix because the sample solution is aqueous.

The base matrix of Sepharose is rigid and do not behave like Sephadex C-25, which is very compressible. Thus, it was previously reported that the Sephadex C-25 beads can be trapped and transferred at low flow rates (less than 20 $\mu l\,s^{-1}$), while at higher flow rates, such as 100 $\mu l\,s^{-1}$, they become squeezed and can flow through the narrow space between the channel and the PEEK tubing stoppers [19]. The rigid property of Sepharose, on the other hand, permits the use of very high flow velocities. No swelling and shrinking were observed at any of the tested pH values. Various flow rates of the solution that passes through the microcolumn filled with the chelating beads were examined, and in no instance were squeezing and leakage of beads encountered from the microcolumn, even at flow rates as high as 200 $\mu l\,s^{-1}$. Hence, the sample loading flow rate for chelating Sepharose can be relatively high in the LOV. The primary advantages of using high flow rate is that high enrichment factors can be attained by using large volumes of sample and that the analysis time can be considerably reduced. As a practical option, a sample flow rate of 50 $\mu l\,s^{-1}$ was employed for further investigations.

In order to remove the remaining non-adsorbed or weakly adsorbed constituents of the matrix in the packed microcolumn after preconcentration a washing step before elution was found necessary. A pH 5.0 buffer was used as the washing solution, since a pH similar to that of the loading sample/NH_4Ac solution will prevent analytes to be eluted prematurely. A 0.01 M solution was found to be satisfactory.

3.3.3. Choice of eluent

Nitric acid and hydrochloric acid with concentrations ranging from 0.1 to 2 M were investigated. Yet, with due consideration to obtaining the lowest reagent blank value and the best elution efficiency, it was found preferable to use nitric acid as eluent. While Cd could be eluted quantitatively with nitric acid concentration ranging from 0.1 to 2 M, Pb and Ni both required the concentration to be at least 1 M. Therefore, 2 M nitric acid was adopted as eluent.

Eluent volumes from 30 to 60 μl were studied. With a volume of 50 μl, virtually quantitative elution of the analyte adsorbed on the packed column was obtained. Smaller volumes revealed non-complete elution and caused decrease of the recorded signal. On the other hand, volumes higher than 50 μl did not give rise to significantly higher signals. Considering the capacity of the graphite tube, a volume of 50 μl eluent was finally used.

Since all operations in the procedure are computer-controlled, the eluent in the tubing system not only can be delivered as a continuously flowing solution forwarded to and through the analyte-loaded column, but also can be stopped within the column itself and remain there for a predetermined time before it is routed to the graphite tube. Thereby, the eluent can obtain sufficient contact time with the beads and facilitate complete dissolution. With this point in mind, an eluent flow rate of 10 $\mu l\,s^{-1}$ and a 5 s stop time within the column, which indeed revealed better eluting efficiency, were adopted for the ensuing analyses.

It was found experimentally that the beads could be used repeatedly up to ca. 20 times with no significant decrease in performance being observable. Therefore, it is only necessary to replace the beads intermittently, as experience dictates.

3.3.4. Measurement of elements

There are, in fact, two possible avenues to measure the metal concentrations after sample loading. One is to transport the analyte-loaded beads directly into the graphite tube, where the beads are pyrolized and the analyte is atomized and quantified. The other one is to elute the analyte-loaded beads with a well-defined volume of eluent and forward the eluate to the graphite tube for determination, the used beads being either discarded or possibly used repeatedly and only discarded intermittently.

In the first approach, any specific requirements to stable surface properties and risks of buildup of back-pressure are completely eliminated. Although having been applied successfully earlier [12], it showed with the application of the chelating Sepharose beads some disadvantages. Thus, a ca. 10% signal decrease compared with the eluting method was observed. Another inconvenience was that after atomization a minute amount of carbon residue remained in the graphite tube, and after repetitive analytical cycles the residue accumulated to a point where the tube needed to be cleaned. Besides, the infection of loaded beads appears to shorten the life-time of the tube. Therefore, the elution approach was found to be preferable for the present procedure.

3.4. Investigation of interferences

Alkaline (K^+, Na^+) and earth alkaline (Ca^{2+} and Mg^{2+}) elements are the most common ones in environmental and biological samples and might potentially interfere with the determination of the target ions. In order to study the interferences effect, 0.01 μg l^{-1} of Cd(II), 0.5 μg l^{-1} of Pb(II) and 0.5 μg l^{-1} of Ni(II) standard solutions with different concentrations of the mentioned ions were analyzed. The maximally tolerated interferent concentrations and interferent/analyte ratios are listed in Table 3. It shows that all three metal ions can tolerate very high concentrations of K^+ and Na^+, but they are somewhat more, as expected, susceptible to interference from the alkaline earth ions, particularly in case of Ni. However, the interferent/analyte ratios were in all cases comfortably high.

3.5. Performance of the SI-LOV system using chelating Sepharose beads

The analytical performance data for the SI-LOV on-line pretreatment ETAAS system are listed in Table 4. The retention efficiency was determined by comparison of the integrated signal obtained after chelating sorption and the one obtained for the total amount of analyte in the sample. The enrichment factor was calculated by comparison with direct of injection of a 50 μl standard solution. The precision was ascertained on the basis of 11 consecutive sample analyses.

The accuracy when using the chelating Sepharose beads was tested by determining the trace level contents of Cd, Pb and Ni in three certified reference materials, that is, CRM 279 (Sea Lettuce), CRM 320 (River Sediment) and SRM 1640 (Natural Water). The experimental results, shown in Table 5, reveal that the values obtained are in good agreement with the certified values, with the ratios between the certified and

Table 3
Tolerances to interferents at 0.01 μg l^{-1} Cd, 0.5 μg l^{-1} Pb and 0.5 μg l^{-1} Ni levels

Interferents	Maximum tolerance (mg l^{-1})			Interferent/analyte ratio		
	Cd^{2+}	Pb^{2+}	Ni^{2+}	Cd^{2+}	Pb^{2+}	Ni^{2+}
K^+	400	400	40	4×10^7	8×10^5	8×10^4
Na^+	200	20	20	2×10^7	4×10^4	4×10^4
Ca^{2+}	0.4	4	0.4	4×10^4	8×10^3	800
Mg^{2+}	0.02	2	0.2	2×10^3	4×10^3	400

Table 4
Analytical performance of the SI-LOV pretreatment system using chelating Sepharose beads for the determination of Cd, Pb and Ni by ETAAS

Parameter	Cd	Pb	Ni
Regression equation	AA = 6.6003[Cd] (μg l^{-1}) + 0.0173	AA = 0.1561[Pb] (μg l^{-1}) + 0.0198	AA=0.2917[Ni] (μg l^{-1}) − 0.0066
Linear range (μg l^{-1})	0.005–0.050	0.10–2.00	0.05–1.00
Bead volume (μl)	20	20	20
Sample volume (μl)	1800	1800	1800
Sample frequency (h^{-1})	12	12	12
Sample loading flow rate (μl s^{-1})	50	50	50
Retention efficiency (%)	95	75	90
Enrichment factor[a]	34	27	32
D.L. (n = 11, μg l^{-1})	0.001	0.07	0.02
Precision (n = 11, %)[b]	3.6	5.1	3.2

[a] Compared with 50 μl direct sample injection.
[b] The concentration of the elements were as follows: C_{Cd} = 0.02, C_{Pb} = 0.50, C_{Ni} = 0.50 μg l^{-1}, respectively.

Table 5
Results of Cd, Pb and Ni determination in certified reference materials

Sample	Certified value	Found value ($n = 4$)
CRM279 ($\mu g\,g^{-1}$)		
Cd	0.274 ± 0.022	0.27 ± 0.01
Pb	13.48 ± 0.36	14.8 ± 0.6
CRM320 ($\mu g\,g^{-1}$)		
Cd	0.533 ± 0.026	0.54 ± 0.02
Pb	42.3 ± 1.6	43 ± 3
Ni	75.2 ± 1.4	76 ± 2
SRM1640 ($\mu g\,kg^{-1}$)		
Cd	22.79 ± 0.96	22.6 + 0.9
Pb	27.89 ± 0.14	30 ± 1
Ni	27.4 + 0.8	26.1 ± 0.9

the found values ranging from 0.9 to 1.10 in the reference materials.

4. Conclusion

On-line pretreatment techniques based on FI/SI/LOV offer great advantages, such as fully automated sample manipulation, low contamination, reduced sample/reagent consumption and waste production.

Chelating Sepharose is a chelating ion-exchanger with iminodiacetate groups as the functional entities, with highly cross-linked hydrophilic agarose as support. It shows excellent chemical and physical stability and allows fast flow rates, and it is therefore ideally suited as adsorbent for use in LOV systems. This was demonstrated by the assay for Cd, Pb, and Ni in three certified reference materials, where the values found were in excellent agreement with the reported values, and where the retention efficiencies were 95, 75 and 90%, respectively.

The proposed SI-LOV system with chelating Sepharose beads possesses the advantages of not only high sensitivity, preconcentration efficiency, repeatability and reproducibility, but also is hydrodynamic impedance free and the beads are easy to handle. Thus, chelating Sepharose serves as a promising separation and preconcentration material useable in fully automatic procedures.

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Paper III

ARTICLE

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An automatic micro-sequential injection bead injection Lab-on-Valve (μSI-BI-LOV) assembly for speciation analysis of ultra trace levels of Cr(III) and Cr(VI) incorporating on-line chemical reduction and employing detection by electrothermal atomic absorption spectrometry (ETAAS)

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A novel, miniaturized micro-sequential injection Lab-on-Valve (μSI-LOV) system hyphenated with electrothermal atomic absorption spectrometry (ETAAS) is proposed for the automatic preconcentration and speciation analysis of Cr(III) and Cr(VI) utilizing solid-phase extraction on hydrophilic chelating Sepharose beads in the renewable bead injection (BI) mode. Exploiting on-line reduction of Cr(VI) to Cr(III), the aspirated sample solution is initially divided into two portions, which are treated simultaneously. Thus, while Cr(III) ions are separated from the matrix constituents, preconcentrated on the beads and subsequently eluted by a small volume of eluent (0.1 mol L^{-1} HNO_3) and quantified by ETAAS, the Cr(VI) ions in the second portion are mixed with a reducing agent and parked under stopped-flow conditions in an open tubular reactor attached to one of the peripheral ports of the LOV unit. Following quantification of the native Cr(III) content, the Cr(III) generated from Cr(VI) plus the original Cr(III) is subjected to the same separation/preconcentration/elution procedure. All sample manipulations are controlled automatically by the integrated software.Under optimized chemical and physical conditions, the flow system, by using a total sample loading volume of 3.6 mL, featured retention efficiencies for Cr(III) as high as 86%, and preconcentration factors of 62 and 42 and detection limits (3σ) of 0.010 and 0.020 μg L^{-1} for Cr(III) and Cr(VI), respectively. The relative standard deviations were 4.7 and 4.5% ($n = 6$) at 0.2 μg L^{-1} for Cr(III) and Cr(VI), respectively, when employing the microcolumn in a renewable fashion, while permanently used sorbent reactors yielded repeatabilities better than 3.0%.The proposed μSI-BI-LOV analyser was successfully applied to the speciation and determination of trace levels of Cr(III) and Cr(VI) in environmental samples. The method was validated by determination of chromium species in CRM and NIST standard reference materials, and by spike recoveries of surface waters. Statistical comparison of means between experimental results and the total chromium certified values for the CRM and NIST materials revealed the non-existence of significant differences at a 95% confidence level.

Introduction

Chemical speciation of metal species has become of major interest in inorganic analysis in the past decades, because of the growing awareness that the toxicity and biological activity of the elements are strongly dependent on their chemical form or oxidation state.[1] Therefore, the accurate determination of the concentrations of the individual species is more meaningful than that of the total element content.

A challenge for analytical chemists in getting knowledge about the natural distribution among defined chemical species is the development of analytical methods preventing the disturbance of the equilibria between the different chemical forms, while ensuring appropriate sensitivity for environmental monitoring. Although atomic spectrometric methods are some of the most sensitive tools for trace metal analysis, the concentration levels of the environmentally relevant analytes to be determined, as required by the regulatory authorities, are often below the dynamic ranges of the instruments. Besides, these detectors are very sensitive to the composition of the matrices. Therefore, a pretreatment step, which can separate the analyte from the matrix components and preconcentrate it before the actual measurement, is often mandatory.

In this context, the second generation of flow injection (FI), the so-called sequential injection (SI),[2,3] should be regarded as a promising tool for automatic sample treatment concerning chemical speciation, separation and preconcentration.[4] Based on the use of a central selection valve, accommodating a number of ports connected to sample, standards, reagents, detector or external units, in turn connected to a high-precision syringe pump serving as an accurate and reproducible liquid driver for solution aspiration and propulsion, it provides an unprecedented versatility for on-line sample manipulations and can readily realize a particular analysis.[5] In comparison with FI systems, which normally operate under a forward-flow regime, the discontinuous programmable flow technique of SI can conveniently achieve automatic solution handling such as flow reversal, stopped-flow, mixing and delivery. Besides, SI provides a plethora of advantages such as simple manifold design, open architecture, robustness and miniaturization,

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which leads to low consumptions of sample and reagents and low production of waste. The potentials of SI for online chemical speciation have been recently summarised by van Staden and Stefan.[6]

The conventional SI system can be micro-miniaturized by using the lab-on-valve (LOV) format.[7] As the name implies, LOV is actually extended to constitute a small laboratory, mounted atop a selection valve, allowing a multitude of unit operations to be executed in an on-line fashion. In our group, LOV has been arranged to admit sorbent materials for solid-phase extraction (SPE)[8] in a bead injection (BI)[9] fashion with the ultimate aim of trace metal separation and preconcentration.[10–12] The hyphenated technique of μSI-BI-LOV has opened new frontiers in analytical chemistry for sample manipulation in trace analysis. In fact, different chemical assays involving reactions either in the liquid or solid phase can be accommodated in the same set-up without re-configuration. Furthermore, the intrinsic miniaturization of the μSI-LOV provides the additional advantage of reduced sample carry-over.[13] However, to the best of our knowledge, no research about speciation procedures in LOV has been conducted so far.

In most flow-injection/continuous-flow chemical speciation publications comprising chemical conversion of one species to another, off-line oxidation or reduction in the batch mode has been used.[14–16] Although the reaction conditions, such as temperature and time, can readily be adjusted to fulfill the required specifications, the risk of sample contamination is much higher when such a procedure is used, and in comparison with an on-line operation mode a considerable amount of labor is often involved. Therefore, on-line conversion of chemical species should prove a promising approach. Thus, one port in the LOV can, for instance, be connected to an open reaction coil without disturbing other operations performed in the miniaturized system, which provides the potential of using the LOV for on-line reaction and on-line preconcentration independently and simultaneously at different peripheral ports.

In this paper, an automated μSI-BI-LOV flow system, applicable to on-line separation, preconcentration and chemical speciation, illustrated by the model of Cr(III) and Cr(VI) species, is described, and proposed as a supplementary approach to the speciated isotope dilution EPA 6800 method,[17] commonly involving anion exchange chromatography in hyphenation with ICP-MS. Exploiting on-line reduction of Cr(VI) to Cr(III) in the conduits of the miniaturized LOV unit, the aspirated sample solution is initially divided into two portions, which are treated simultaneously, as depicted in Fig. 1. Thus, while Cr(III) ions are separated from the matrix constituents and preconcentrated on hydrophilic chelating Sepharose beads, subsequently eluted by a small volume of eluent (25 μL of 0.1 mol L^{-1} HNO_3) and quantified by ETAAS, the Cr(VI) ions in the second portion are mixed with a reducing agent and parked under stopped-flow conditions in an open tubular reactor attached to one of the peripheral ports of the LOV unit. Following quantification of the native Cr(III) content, the Cr(III) generated from Cr(VI) plus original Cr(III) are subjected to the same separation/preconcentration/elution procedure.

Chromium exists mainly in the hexavalent and trivalent states. In the environment Cr(III) occurs as $Cr(OH)_n^{(3-n)+}$, and Cr(VI) as CrO_4^{2-} or $HCrO_4^-$, although Cr(III) normally dominates, because Cr(VI) is easily reduced by organic substances. Chromium is commonly present in soils, waters, rocks, fauna and flora, and in volcanic dust and gases. The occurrence of chromium results mainly from human activities *via* production of waste water by the metallic smelting, electroplating, hide processing and dyestuff industries. The biological and chemical properties of the two species differ significantly. Thus, Cr(VI) compounds are approximately 100 times more toxic than Cr(III) salts, due to their high oxidation potentials and the ease with which they penetrate biological membranes.[18] Yet, Cr(III) is an essential trace element in the human body and it is important in the metabolism of glucose and certain lipids such as cholesterol.[19] Consequently, the speciation analysis of Cr(III)/Cr(VI) is of great importance and, therefore, much research effort has been devoted to this area.

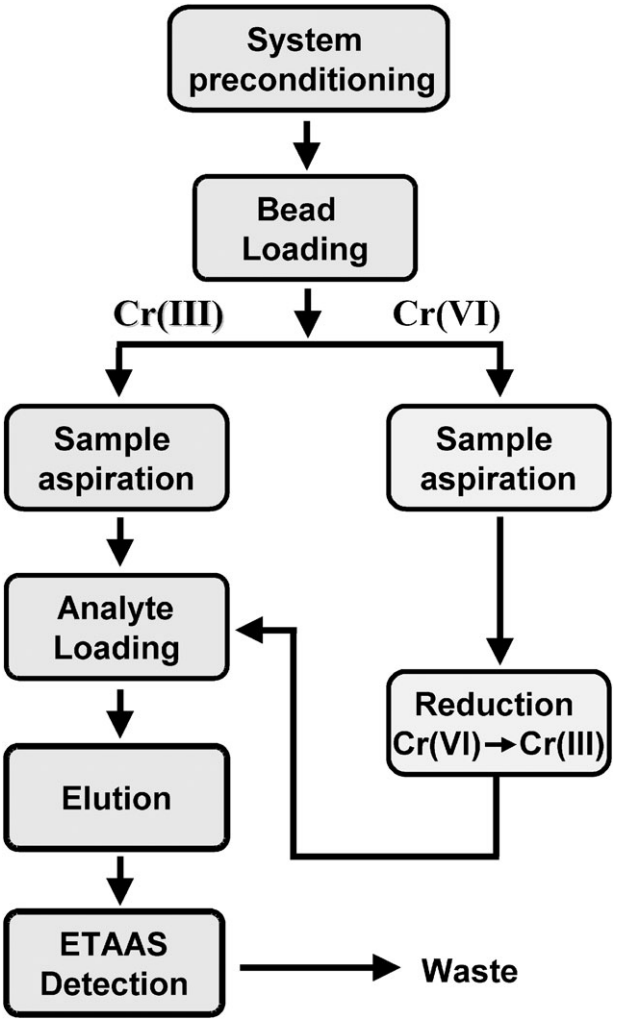

Fig. 1 Flow chart of the SI-procedures to which the Cr(III) and the Cr(VI) species, present in the original sample solution, are subjected. While the Cr(III) ions are separated/preconcentrated on the chelating Sepharose beads and subsequently eluted and quantified by ETAAS, the Cr(VI) ions are reduced to Cr(III) by hydroxylamine (in an open reaction coil, as shown in Fig. 2), and afterwards treated as the native Cr(III) ions.

Experimental

Instruments

A PerkinElmer AAnalyst 600 atomic absorption spectrometer with a Zeeman background corrector, an AS-800 autosampler, and a transversely heated graphite furnace equipped with pyrolytically coated graphite tubes was employed for all the measurements. A chromium hollow cathode lamp (PerkinElmer) was used at a wavelength of 357.9 nm and a spectral bandpass of 0.7 nm and was operated at a current of 25 mA. The temperature program for the chromium analysis is listed in Table 1. The signals were recorded in the integrated (peak area) mode.

The sequential injection system (FIAlab-3000, Bellevue, WA) consists, as depicted in Fig. 2, of two high-precision bi-directional syringe pumps (SP1 10 mL, SP2 2.5 mL), a peristaltic pump, and a six-port selection valve (SV) mounted with the integrated LOV central sample-processing unit (details of which are given below), which connects to a holding coil (HC) and a reaction coil (RC). The two-way valves at the heads of

Table 1 Temperature program for the ETAAS determination of chromium

Step	Temperature/°C	Ramp time/s	Holding time/s	Argon flow rate/mL min^{-1}
Drying 1	110	5	20	250
Drying 2	140	5	40	250
Pyrolysis	1500	10	20	250
Atomization	2300	0	4	0
Cleansing	2450	1	3	250

SP1 and SP2 facilitate the communication of each syringe with either an external reservoir (carrier or reducing agent) or with the manifold. A PEEK T-connector was used for assembling the conduits of both syringes to the SV. Both HC and RC have a volume of 2.5 mL and were made from PTFE tubing with a length of 500 cm and diameters of 0.80 mm id/1.60 mm od, and a length of 320 cm with 1.00 mm id/2.00 mm od, respectively. Other connecting tubing was made from 0.50 mm id/1.66 mm od PTFE tube. The peristaltic pump, connected to the dual-access port 5, was furnished with Tygon tubing of 1.22 mm id/2.80 mm od, allowing for a flow rate of 2.1 mL min^{-1}, and was employed to fill the conduits with fresh sample solution when switching samples, thereby minimizing any risks of sample carryover.

The LOV microsystem (diameter: 5 cm, thickness: 1 cm), made from hard PVC, contains six micro-channels (1.66 mm id/12.0 mm length) which can be made to communicate with the central port through the central communication conduit (CC) in the SV. The two channels connecting the central port and port 4 serve as positions for bead-microcolumns C_1 and C_2, in which PEEK stoppers (Upchurch Scientific, Oak Harbor, WA), which have a dimension slightly smaller than that of the channel, are used for retaining the beads while allowing the solution to flow freely.

The operational sequences of the μSI-BI-LOV system were controlled by a dedicated computer program (FIAlab, USA) and synchronised with the commands of the AAWinLab Analyst detector software (PerkinElmer). Hence, just before starting the elution step, the ETAAS program was activated through an intelligent electronic interface.[20] During the run of the latter ETAAS measuring sequence, the μSI-BI-LOV method was again commenced to simultaneously execute the analytical protocol for the next analysis cycle.

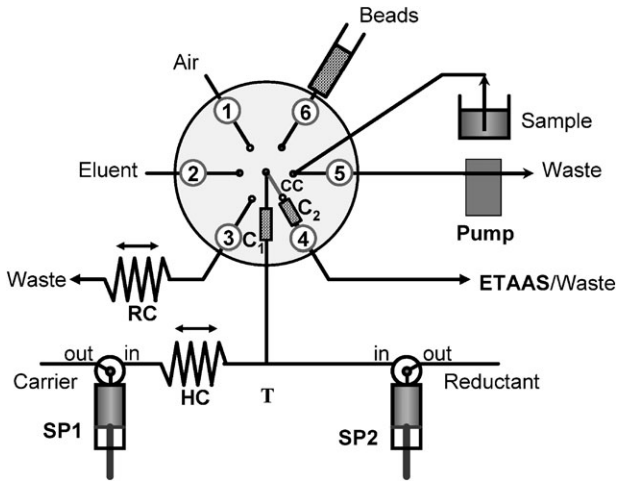

Fig. 2 Schematic drawing of the μSI-BI-LOV-ETAAS system for Cr(III) and Cr(VI) speciation. Carrier, 0.005 mol L^{-1} formic acid/formate buffer at pH 3.8; reductant, 0.02 mol L^{-1} hydroxylamine in 0.005 mol L^{-1} pH 3.8 buffer; eluent, 0.1 mol L^{-1} HNO_3; beads, Chelating Sepharose; SP1/SP2, syringe pumps 1 and 2; C_1 and C_2, LOV microcolumn positions; HC, holding coil; RC, reaction coil; CC, central communication channel; ETAAS, electrothermal atomic absorption spectrometric instrument; T, merging point (T-piece).

The pH of all solutions were measured by a digital pH meter (PHM92 LABpH Meter, Radiometer Danmark A/S).

Reagents and solutions

Doubly de-ionised water (18.2 MΩ cm) obtained from a Milli-Q system (Millipore Synthesis A10, France) was used throughout for preparation of the various solutions. A 0.005 mol L^{-1} formic acid/formate buffer of pH 3.80 ± 0.05—which matches the pK_a value of formic acid—was prepared by dissolving 0.3400 g of sodium formate in 1000 mL of water and adjusting the pH by dropwise addition of 1.0 mol L^{-1} HNO_3. This solution was used for preparing carrier, standards, samples and reducing agent. Cr(III) and Cr(VI) working standard series were obtained by appropriate dilution of a 1000 mg L^{-1} of chromium(III) nitrate and potassium chromate standard solutions (Merck) in the pH 3.8 buffer. Mixed standards of Cr(III) and Cr(V) were used for sample speciation. A 0.02 mol L^{-1} hydroxylamine solution was prepared by dissolving 0.1398 g of hydroxyammonium chloride in 100 mL of the pH 3.8 buffer. The eluent was obtained by a 10-fold dilution of a 1.0 mol L^{-1} HNO_3 stock solution in Milli-Q water.

All flasks and beakers for solution preparation were cleansed with a 25% (v/v) concentrated nitric acid–water solution utilising a washing machine (Miehle, Model G 7735 MCU, Germany) followed by repeated washing with Milli-Q water.

Commercially available agarose-based Chelating Sepharose™ Fast Flow beads (Amersham Biosciences, Sweden), with covalently immobilised iminodiacetate moieties and nominal bead size of 90 μm (distribution range of 45–165 μm), were received and stored in 20% ethanol solution and used directly with no additional treatment, being contained in the syringe mounted atop port 6 of the LOV which was acting as the bead reservoir.

Sample pretreatment

The reference materials employed were SRM 1640 (Natural Water) from The National Institute of Standard and Technology (NIST) and CRM 320 (River Sediment) from The Community Bureau of Reference. The liquid SRM 1640 was diluted in the pH 3.8 buffer directly before measurement. The solid sample was digested according to the following procedure: 0.5 g of CRM 320 was weighed in PTFE vessels. Three replicates of the sample plus two blanks were moistened with 1 mL of water before adding 3 mL of nitric acid (65%) and 9 mL of hydrochloric acid. The solution was kept at room temperature for 2 hours and then gently heated to near dryness in a sand bath with the temperature not exceeding 140 °C. After cooling, 3.0 mL of hydrofluoric acid (40%) was added. The sample was then heated to near dryness again. After the sample had cooled down, 2 mL of perchloric acid was added, whereafter the sample was again heated to near dryness. Finally, 0.5 mL of 65% nitric acid and 20 mL of water were added to the residue and the solution was heated to near the boiling point. Upon cooling down the solution was transferred to a 100 mL volumetric flask and diluted to the mark with water. Further appropriate dilution in the pH 3.8 buffer was carried out to keep the analyte concentration within the linear dynamic range before measurement.

The environmental water samples were, after sampling, filtered by using a 0.45 μm pore size membrane filter and analyzed without delay. The tap and river waters were diluted 10 times in the pH 3.8 buffer to prevent matrix interferences on the sorptive separation system caused by the high concentration of concomitant alkaline earth metals, while assuring detectable concentrations of chromium species for the μSI-LOV-ETAAS analysis.

Procedure

The μSI-BI-LOV protocol consists initially of system preconditioning and then of aspiration of two portions of sample solution. One portion of the sample is destined for Cr(VI) reduction, while the other portion is directly subjected to analyte separation and preconcentration, elution and ETAAS measurement of Cr(III) (see Fig. 1). The two procedures are executed simultaneously. Finally, the reduced fraction is submitted to a similar handling as the native Cr(III). The detailed operational sequences of a complete measuring cycle for chromium speciation are listed in Table 2 and summarized as follows.

System preconditioning (step 1). Before running the speciation program in the SI system, the line connecting the eluent reservoir (port 2) with the LOV, the one connecting SP2 with the T-connector and that connecting SP1 and HC are washed and filled with eluent (0.1 mol L^{-1} HNO_3), reductant (0.02 mol L^{-1} hydroxylamine) and carrier (pH 3.8 buffer), respectively. Carrier solution and reducing reagent are aspirated by SP1 and SP2 from their respective external reservoirs for subsequent use. Then an air spacer is introduced before 400 μL of eluent is directed from the reservoir to the HC. A 25-μL portion of chelating Sepharose beads is aspirated into column position C_1, subsequently moved to column position C_2 and rinsed with 400 μL of acid and then with 300 μL of carrier. The RC is washed with 800 μL of carrier and the mixing point of the T-connector is flushed by 100 μL of reducing reagent. This rinsing protocol assures absorbance blank levels ≤ 0.05 in the enclosed system, the major contribution being the contamination of carrier and reagent solutions by traces of chromium.

Table 2 Assay protocol of μSI-BI-LOV-ETAAS for speciation analysis of Cr(III) and Cr(VI)

Step	SP1 valve[a]	SP2 valve[a]	SP1	SP2	LOV position	Flow rate/ μL s^{-1}	Volume/ μL	Remarks
1								System preconditioning
	Out	Out	Aspirate	Aspirate	—	100(SP1) 50(SP2)	2790(SP1) 750(SP2)	(a) Filling of SP1 and SP2 with carrier and reductant, respectively
	In	Out	Aspirate	—	1	50	100	(b) Introduction of air segment
	In	Out	Aspirate	—	2	50	400	(c) Aspiration of 0.1 M HNO_3 into HC
	In	Out	Aspirate	—	6	5	25	(d) Collection of beads into C_1
	In	Out	Dispense	—	4	10	425	(e) Transportation of beads to C_2 and acid cleansing
	In	Out	Dispense	—	4	50	400	(f) Washing of beads and ETAAS line with carrier
	In	In	Dispense	Dispense	3	50(SP1) 20(SP2)	800(SP1) 100(SP2)	(g) Washing of RC and mixing point
2								Sample preparation for Cr(VI) reduction
	In	In	Aspirate	—	1	50	100	(a) Air segment introduction
	In	In	Aspirate	—	5	100	1800	(b) Loading of sample into HC
	In	In	Aspirate	—	1	50	110	(c) Air introduction for compensating the dead volume between mixing point and LOV
	In	In	Dispense	Dispense	3	60(SP1) 15(SP2)	2050(SP1) 550(SP2)	(d) Merging sample with reductant and transport into RC
3								Cr(III) preconcentration
	In	In	—	Aspirate	1	30	120	(a) Air introduction into HC and SP2 line
	In	In	Aspirate	—	5	100	1800	(b) Loading of sample into HC
	In	In	Dispense	—	4	75	2300	(c) Cr(III) preconcentration and column clean-up
4								Filling of ETAAS line with air
	In	In	Aspirate	—	1	50	760	(a) Introduction of air into HC
	In	In	Dispense	—	4	20	380	(b) Filling of ETAAS line with air
5								Activation of ETAAS
6								Elution for Cr(III) measurement
	In	In	Aspirate	—	2	10	25	(a) Aspiration of eluent
	In	In	Dispense	—	4	10	430	(b) Elution of analyte loaded beads
7								Delay 60 s to prevent overlapping between consecutive ETAAS runs
8								Total Cr(III) preconcentration
	In	In	Dispense	—	4	50	300	(a) Washing of beads
	In	In	Aspirate	—	3	75	2500	(b) Aspiration of reduced sample solution into HC
	In	In	Dispense	—	4	75	3000	(c) Analyte loading and column clean-up
9								Filling of ETAAS line with air
	In	In	Aspirate	—	1	50	760	(a) Introduction of air into HC
	In	In	Dispense	—	4	20	380	(b) Filling of ETAAS line with air
10								Activation of ETAAS
11								Elution for total Cr measurement
	In	In	Aspirate	—	2	10	25	(a) Aspiration of eluent
	In	In	Dispense	—	4	10	430	(b) Elution of analyte loaded beads
12								Bead discarding
	In	In	Dispense	—	4	100	300	(a) Dispensing of carrier to C_2
	In	In	Aspirate	—	1	100	300	(b) Bead transportation from C_2 to C_1 positions
	In	In	Dispense	Dispense	3	50(SP1) 20(SP2)	300(SP1) 120(SP2)	(c) Delivery of beads to waste

[a] The position "out" means connection of SP with the external reservoir, while "in" means connection of SP with the manifold.

Sample preparation for Cr(VI) reduction (step 2). Following the introduction of an air segment, the selected (1.8 mL) portion of sample for the Cr(VI) assay is aspirated into HC. Then, 110 μL of air is aspirated to fill the line connecting the T-connector with the LOV in order to ensure that the head of the sample plug is positioned behind the mixing point (T). SP1 and SP2 are then activated simultaneously to drive the combined solutions of the sample and reducing reagent plugs through port 3 and into the RC, where the mixture is stopped and stacked for the duration of the reduction reaction.

Cr(III) preconcentration, elution and measurement (step 3–7). Firstly, a minute air plug is aspirated to fill the connecting line between the LOV and the T-connector and a fraction of the line connecting to SP2 in order to separate the forthcoming sample from the reducing reagent. After the 1.8 mL portion of the sample has been aspirated into HC, the communication channel is then connected to the peripheral port 4. The sample is passed through the sorbent in column C_2. As a result, Cr(III) is retained on the beads by chelation. An extra amount of 400 μL of the previously stored carrier solution is used to rinse the analyte loaded sorbent and remove the weakly or non-retained matrix constituents on it.

The remaining solution in the ETAAS line is replaced by an air zone. Then, 25 μL of 0.1 mol L^{-1} HNO_3 eluent are aspirated into HC, the ETAAS program is activated and the autosampler tip is inserted into the dosing hole of the graphite tube. The air sandwiched eluate segment is immediately forwarded to the graphite tube and the Cr(III) signal obtained upon completion of the ETAAS temperature program.

Total Cr preconcentration, elution and measurement (step 8–11). While the Cr(III) content in the sample is being determined, the μSI-LOV protocol for the total chromium assay is initiated. To this end, the beads remaining in C_2 are rinsed by 300 μL of carrier, whereupon the reduced portion of the sample in RC is directed backwards to HC and then forwarded to pass through microcolumn C_2. After sample loading, a procedure similar to that depicted in steps 3–7, comprising washing step, air sandwiched elution and ETAAS activation and measurement, is effected. Thereby, a signal corresponding to the total Cr content in the sample is obtained.

Beads removal (step 12). If called for, the used beads can be discarded by transferring them from C_2 to C_1 with carrier and then further delivering them to waste through port 3. This strategy was adopted in this work for speciation analysis of chromium in real-life samples.

Results and discussion

Configuration of the μSI-BI-LOV system for chemical speciation

Most of the flow systems for chemical speciation of Cr(III) and Cr(VI) involve on-line separation and derivatization of specific chemical forms into detectable products prior to detection. Single column SPE methods for separation and preconcentration have been commonly exploited, in which one target species is isolated and measured on-line while the other one is calculated on the basis of the total concentration, as determined by a similar procedure following batch reduction or oxidation of the sample. Therefore, full automation is not accomplished. There is only one report employing a single column in a sequential injection network, in which Cr(III) and Cr(VI) species are simultaneously retained on the same sorbent material (*viz*., activated alumina) and sequentially eluted.[21] Yet, a significant drawback of this procedure is that it requires multiple elutions, thereby yielding poor enrichment factors.

Another way is to incorporate into the flow network two types of solid-phases in a tandem or parallel fashion, packed with anion and cation exchangers, for the retention of Cr(III) and Cr(VI) species, respectively.[22–24] However, the impossibility of adjustment of optimum loading pH for both sorbents often results in a compromise choice of the sample pH, which is not necessarily the best for any of them. Besides, the arrangement of two permanent packed columns in series in unidirectional flow assemblies usually results in impaired performance as a consequence of the build-up of back-pressure, which severely affects the long-term operation of the flow system.

To alleviate the aforementioned drawbacks, our method makes use of two portions of the same sample solution that are simultaneously processed in an automated manner within the same manifold using a single solid phase of renewable nature. For on-line Cr(VI) reduction, two syringe pumps merging in a T-confluence are simultaneously activated, the main one being used to deliver the sample while the auxiliary one propels a defined zone of reducing agent (see Fig. 2). As opposed to the limited interdispersion when stacking zones in tandem in the conventional SI mode, the hybrid FI-SI protocol presented herein assures efficient radial mixing between sample and reductant zones in all elements of the combined solution.

Regarding the dimensions of the open RC, it was experimentally found that tubing diameters ≤ 1.0 mm od and the use of knotted reactors were not an advantage in the present system despite their recognized capabilities for improvement of solution mixing, because they hampered the efficient backward aspiration of delivered sample and reductant zones.

The use of air segmentation in the proposed system is most beneficial in defining the sample zone and acid plugs and preventing undue dispersion, which is common in FI/SI between two neighboring zones. Thus, as can be seen from the operational procedure, the preconditioning step comprises the introduction of air bubbles between the carrier and the acid. If this were not implemented, the presence of acid within the carrier, as caused by dispersion, would remain at the far end of the HC and eventually pass through the analyte loaded beads during the washing step following sample loading, resulting in analyte breakthrough owing to the pH gradient in the carrier stream. Air spacers are also used to form discrete sample zones, whereby the portion for reduction or the portion only for Cr(III) assay can be transported from one conduit to another without taking dilution effects into consideration. Besides, air sandwiched eluates are carried to the graphite furnace so as to fulfill the volume requirements of the atomizer.

Investigation of chemical variables

Chelating Sepharose was chosen as a hydrophilic sorbent material because of the high retention efficiencies and fast adsorption rates for many transition metals.[12] Besides, it is ideal for the operation in the LOV mode, that is, the sorptive entities are easily manipulated within the microbore conduits because of their perfectly spherical shape and appropriate particle size distribution. Furthermore, the chelating solid-phase admits high flow rates during the sample loading step, and, as opposed to dextran-type Sephadex resins, the extent of swelling/shrinking upon pH change is negligible as a consequence of the highly cross-linked agarose matrix.

A prerequisite for the realization of on-line Cr(VI) reduction is the selection of a chemical reductant facilitating fast reaction development. Different strong organic and inorganic reductants such as ferrous iron,[25] potassium sulfite,[26] hydroxylamine,[14,27–29] hydrogen peroxide[30,31] and ascorbic acid,[15,16,32,33] mainly in acidic medium, have been reported. The issue of suitability for the on-line μSI sorbent extraction assay is addressed in Fig. 3. Ascorbic acid has been regarded as the most ideal reductant in several publications due to its high reaction rates and improved reduction efficiency. The

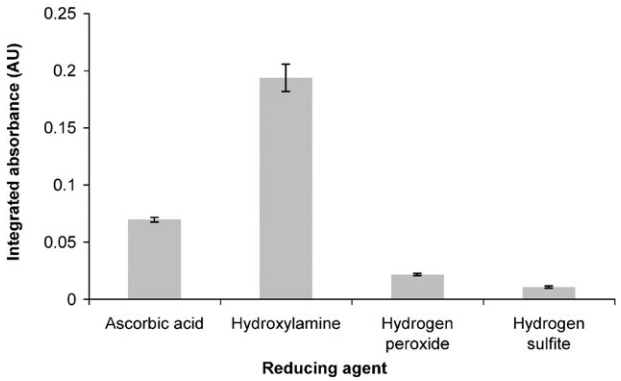

Fig. 3 Comparison of the effectiveness of different kinds of reducing reagents for on-line conversion of Cr(VI) into Cr(III) at pH 4.0. All the reducing agents are prepared at the 0.05 mol L^{-1} level. Sample loading volume: 1.8 mL; sample to reductant volume ratio: 4 : 1; eluent volume: 25 μL; Cr(VI) concentration: 0.4 μg L^{-1}; reduction time: 180 s.

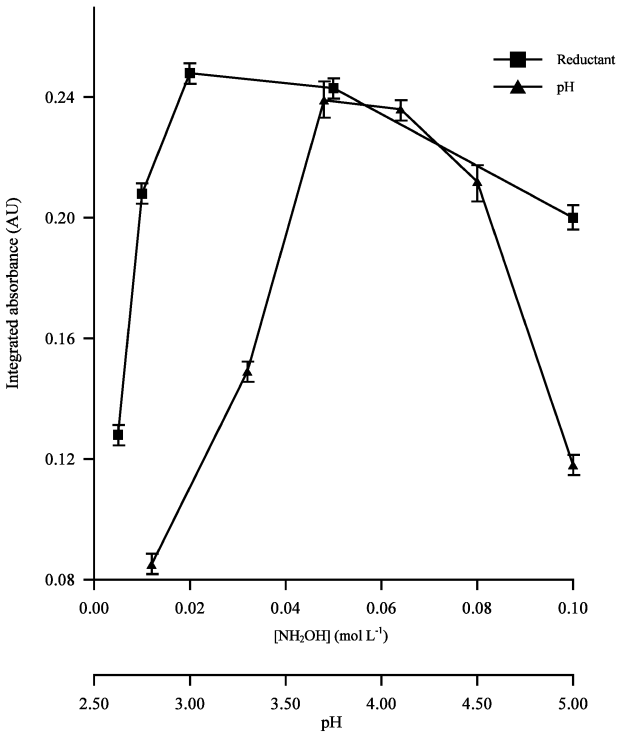

Fig. 4 Effect of the nominal concentration of hydroxylamine (■) and pH (▲) on the reduction of Cr(VI) to Cr(III) and subsequent preconcentration of Cr(III). Sample loading volume: 1.8 mL; sample to reductant volume ratio: 4 : 1; eluent volume: 25 μL; Cr(VI) concentration: 0.4 μg L^{-1}; reduction time: 240 s.

experimental results from different research groups revealed some discrepancies concerning both reaction time and efficiency,[15,32] but they all reported that ascorbic acid can reduce Cr(VI) nearly quantitatively within minutes. However, when it was employed in our preconcentration system at the 0.05 mol L^{-1} level with on-line reduction, or even in the off-line reduction mode, the recovery of chromium was only 20% under mild reaction conditions, *i.e.*, pH 4.0. Even when the reaction time was increased to 30 min no results similar to those published previously[15,32] were obtained. This is probably due to the fact that the former authors monitored the fading of the Cr(VI) solution spectrophotometrically, while our μSI-LOV system deals with Cr(III) species, and only the free or labile Cr(III) can be retained on the sorbent and therefore determined. Further experiments in which a Cr(III) standard was pre-mixed with reductant and passed through the sorbent showed that ascorbic acid did not affect the retention yields of Cr(III) on chelating beads. This, in turn, proved that if only ascorbic acid and Cr(III) are present no chelate compound is formed, instead an inert dehydroascorbic acid (DA) complex of Cr(III) is generated during the reduction process of Cr(VI),[27] which makes the ligand exchange of DA with the iminodiacetate moieties of the beads incomplete.

Hydrogen peroxide in acidic medium (0.05 mol L^{-1}; pH = 4.0) provided less than 10% reduction efficiency and poor reproducibility due to the vapor bubbles formed in the miniaturized system, which made the on-line aspiration and delivery of the reagent troublesome. Hydrogen sulfite virtually did not reduce Cr(VI) at all under similar chemical conditions. Metallic reducing reagents, such as ferrous iron, were deliberately avoided in the system to prevent deterioration of the enrichment procedure, because iron can also be sorbed by chelation.

On the other hand, preliminary batch experiments confirmed that Cr(VI) was quantitatively reduced by hydroxylamine (0.05 mol L^{-1}; pH = 4.0) at room temperature without further disturbance of the sorption process. To optimize the hydroxylamine concentration for the automated Cr(VI) reduction, standards containing 0.4 μg L^{-1} Cr(VI) were merged on-line with various solutions of reducing agent, with nominal concentrations ranging from 0.005 to 0.1 mol L^{-1} at pH 4.0, using a reduction time of 4 min. According to the results shown in Fig. 4, the highest reduction efficiency is obtained around a concentration of 0.02 mol L^{-1}, which corresponds to an effective reductant to analyte concentration ratio of 6.5×10^5, and this concentration was finally chosen for further research. Lower concentrations did not expedite the redox reaction while higher concentrations gave rise to a higher reagent blank. The recovery yields decreased slightly above a concentration of 0.05 mol L^{-1} NH_2OH, which is attributed to the pre-elution of Cr(III) caused by electrostatic interactions between the iminodiacetate groups and the excess reductant, which is protonated at pH 4.0. Since the SP2 driven reductant is mixed with the sample zone at a 1 : 4 flow-rate ratio, a concentration of 0.02 mol L^{-1} NH_2OH in the reductant reservoir corresponds to a concentration of 0.004 mol L^{-1} in the reaction medium.

The reduction rate of hydroxylamine was also investigated. It was found that within the first 1–2 min the Cr(VI) reduction is very fast, until a plateau level is reached after 4 min. The contact time between sample and reductant in the μSI-LOV system during the treatment of the sample portion for Cr(III) determination is *ca.* 4.0 min (Table 2), which satisfies exactly the temporal requirements of the on-line redox process. Although this time does not result in quantitative conversion (see Table 3), it was experimentally verified by testing various Cr(III) to Cr(VI) concentration ratios that the conversion efficiency remained virtually constant.

The pH of the reductant is a key factor, not only in respect to the reaction development rate but also because the sorption of Cr(III) on the chelating surfaces is strongly dependent on it. The more acidic the medium is, the more beneficial for the reduction. The widely used pH for quantitative conversion of Cr(VI) into Cr(III) by hydroxylamine is 2.0 ± 0.2.[27–29] Yet, the best retention yields for Cr(III) are attained above pH 3.5. At a lower pH the functional groups of the chelating Sepharose are protonated, thus hindering the sorptive preconcentration of Cr(III), while at milder acid or alkaline conditions hydrolysis of the metal ions occurs, making them inaccessible for the sorptive material. Therefore a compromise between the reduction of Cr(VI) and the chelation of Cr(III) must be made. Fig. 4 shows the analytical signals recorded after on-line reduction and preconcentration of chromium species at different sample pH-values. As can be seen, the highest sensitivity is obtained within the pH range of 3.5–4.0. Therefore, the reducing agent and carrier solution were adjusted to a final pH of 3.8. For the samples, this pH adjustment was done immediately before injection into the flow set-up to minimize re-distribution between oxidation states.

Table 3 Analytical performance of the μSI-BI-LOV-ETAAS system using on-line reduction for Cr(III) and Cr(VI) speciation

Parameter	Cr(III)	Cr(VI)
Regression equation	1.0792 [Cr(III), μg L^{-1}]	0.7380 [Cr(VI), μg L^{-1}] – 0.0008
Correlation coefficient	0.9988	0.9990
Linear range/μg L^{-1}	0.02–0.28	0.035–0.4
Sample volume/mL	1.8	1.8
Loading flow rate/mL min^{-1}	4.5	4.5
Maxium injection throughput/h^{-1}	12	8
Eluent volume/μL	25	25
Retention efficiency (%)	86	—
Reduction efficiency (%)	—	68
Enrichment factor	62	42
Concentration efficiency/min^{-1}	12.4	5.6
Detection limit/μg L^{-1} (3σ)	0.010	0.020
Repeatability (%, 0.2 μg L^{-1}, $n = 7$)	2.4	2.2
Reproducibility (%, 0.2 μg L^{-1}, $n = 6$)	4.7	4.5

Formic acid–sodium formate was chosen as the buffer, because it has no complexing capabilities for Cr(III) and it has a similar pK_a-value to the optimized pH, whereby maximum buffer capacity is ensured. A concentration of buffer higher than 0.01 mol L^{-1} is not recommended in our system because of the surplus of competitive sodium ions for the active sites of the sorbent. Therefore, a 0.005 mol L^{-1} buffer concentration at pH 3.8 was used in the preparation of the various solutions.

Investigation of μSI-BI-LOV variables

To achieve the best performance for the separation and preconcentration of Cr(III) in the designed μSI-BI-LOV system, physical parameters related to sample loading and elution of sorbed species were optimized.

A high flow rate of sample can greatly reduce the loading time through the beads in the column, especially when a large sample volume is used to obtain a high enrichment factor, by which an improved overall sample throughput can be obtained. However, the kinetics of chelation must be taken into consideration. To this end, loading flow rates ranging from 25 to 125 μL s^{-1} were assayed. Experimental results showed that the sensitivity did not decrease significantly at a sample flow rate as high as 75 μL s^{-1}. The integrated absorbance for identical concentrations only had a 7% decrease at 75 μL s^{-1} as compared with that of 25 μL s^{-1}, while a flow rate of 100 μL s^{-1} gave rise to a 10% signal deviation. A sample loading rate of 75 μL s^{-1} was hence chosen for further investigation. Very importantly, at this sample flow rate no significant back pressure was encountered and no bead leaking by squeezing through the space between the PEEK stopper and the column channel wall took place.[12]

Nitric acid was selected as the eluent to strip the target Cr(III) ions out from the chelators by protonation of the iminodiacetate groups. Regarding eluent concentrations, 0.01–2 mol L^{-1} HNO_3 levels were tested and it was found that, in comparison with the need for using high concentrations of nitric acid (2 mol L^{-1}), as required for other metal ions (*e.g.*, Cd, Ni and Pb),[12] the inert Cr(III) ions are weakly adsorbed on the beads and a concentration of 0.1 mol L^{-1} sufficed for quantitative elution of the analyte. A relatively low concentration of acid will benefit the instrumental system and the laboratory operation, besides providing a low blank signal. Therefore, a concentration of 0.1 mol L^{-1} HNO_3 was finally adopted.

A small volume of eluent is preferable for the ETAAS, not only because the graphite tube has a limited capacity, but also a higher enrichment factor can thus be obtained. The volume of eluent is related to the strength of the adsorption of the analyte, the nature of the beads and the column dimensions. Compared with the external packed column mode,[16,21,34] the micro-column integrated with the LOV often requires a smaller volume of eluent for effective stripping of preconcentrated species. In the present system a single 25 μL-plug of eluent proved sufficient to elute the analyte quantitatively and reproducibly and was hence used.

Different eluent flow rates were investigated for the air sandwiched elution modality. Cr(III) could be readily eluted by flow rates ranging from 5 to 20 μL s^{-1}. However, in order to prevent multiple segmentation of the discrete eluate zone and facilitate reliable introduction of the enriched segment into the graphite tube platform without liquid spattering, flow rates lower than 20 μL s^{-1} should be employed. Hence, a flow rate of 10 μL s^{-1} was selected for the remainder of the studies.

Interferences

The effect of foreign ions on the solid-phase extraction/preconcentration of Cr(III) was evaluated using a fixed concentration of 0.2 μg L^{-1} Cr(III) and variable amounts of potentially interfering ions. A given concentration of a chemical species was regarded as an interferent whenever the analytical readout varied more than 10%. The most ubiquitous foreign ions, such as K(I), Na(I), Ca(II) and Mg(II), and also common environmentally relevant ones, such as Cd(II), Pb(II) and Ni(II), and trivalent ions Fe(III) and Al(III), were investigated and the results are listed in Table 4. Compared with a recently described FI-speciation system,[34] the proposed μSI-LOV method with renewable surfaces has a much higher tolerance to foreign ions, *i.e.*, it can tolerate 250, 100, 50, 25, 25 and 20 times higher concentrations of Ni(II), Pb(II), K(I), Cd(II), Cu(II) and Fe(III), respectively, with no need for masking agents. Even when using complexing agents, such as fluoride, glycine and tartrate, the FI system exhibits impaired tolerance levels for given species. Thus, for example, the LOV method proposed herein still has 50 and 2.5 times higher tolerance to Ni(II) and Cu(II). The reason probably lies in the fact that both Cr(III) and other transition metal ions can be retained on the surface of the beads, but Cr(III) can be more easily released at the eluent concentration of 0.1 mol L^{-1} nitric acid than other transition/heavy metals, which require an acid concentration ranging from 1 to 2 mol L^{-1} for quantitative elution.[12] The introduction of cleaner eluates into the graphite furnace, in turn, results in less interfering effects during atomization from concomitantly preconcentrated metal ions.

To eliminate the cumulative interfering effect of metal ions remaining on the sorbent, the beads should be renewed after each sample analysis. This is especially necessary for environmental samples such as underground waters, which sometimes contain high concentration of electrolytes. But for clean samples, it is feasible to use the micro-column in a permanent

Table 4 Tolerance of the proposed preconcentration method to potentially interfering species[a]

Foreign species	Tolerated interferent/Cr(III) ratio
K(I)	2×10^6
Na(I)	1×10^6
Ca(II), Mg(II), Cd(II), Pb(II), Ni(II)	5×10^4
Cu(II)	5×10^3
Fe(III), Al(III)	5×10^2

[a] [Cr(III)]: 0.2 μg L^{-1}.

fashion, the beads only being replaced when necessary. In our experiments it was found that the same column could be used for at least 40 standard injections (40 × 1.8 mL) without loss of performance.

The synergistic effect between both chromium valence states was studied by analysing a sample with a fixed Cr(III) concentration (0.2 μg L^{-1}) and different concentrations of Cr(VI). As opposed to previous works reporting Cr(VI)/Cr(III) tolerance ratios as low as 2,[34,35] no significant re-distribution between species was detected in the proposed method for concentration ratios ≤10 when using calibration curves obtained from mixed standard series.

Analytical performance of the μSI-BI-LOV system for Cr(III) and Cr(VI) speciation. The merits of the μSI-BI-LOV system for Cr(III) and Cr(VI) speciation, when operated under the optimized parameters, are listed in Table 3. The calibration lines for Cr(III) and Cr(VI) were obtained simultaneously by using mixed standard series of both oxidation states. The detection limit was calculated as the concentration of analyte providing a signal equal to three times the standard deviation of the blank. The enrichment factor is defined as the ratio between the sensitivity of the proposed method and that obtained by direct injection of 25 μL of a series of standards. The retention efficiency is calculated as the ratio of the sorbed amount of analyte to the total amount available for the chelating moieties. The concentration efficiency is given as the product of the enrichment factor and the sampling frequency per minute. The repeatability and reproducibility correspond to the precision obtained in application of the permanent and renewable column modes, respectively. The maximum injection throughput is given by single species measurements. The reduction rate is calculated as the ratio of the linear range sensitivity for Cr(VI) after both on-line reduction and preconcentration to that of Cr(III) after solid-phase enrichment.

As compared with a flow-injection Cr-speciation procedure based on the sequential use of two permanent columns mounted at the tip of the ETAAS autosampler arm,[34] the proposed LOV approach shows significant advantages in every analytical feature but the sample throughput. The sensitivity, enrichment factor and detection limits for both Cr(III) and Cr(VI) are almost 10-fold better than they are in the alternative method using two extraction microcartridges. Besides, the retention efficiency for Cr(III) is *ca.* 6 times higher. The excellent performance of the fully automated system results from the ideal sorbent material adopted in the microcolumn. On the contrary, the column-in-tip method is only operating in a semi-automatic mode. In fact, following the determination of a single compound, the sorbent column must be manually removed and replaced by another one, aiming to accomplish the speciation protocols. So only at the ideal conditions, and with experienced operation, can the nominal analytical throughput be realized.

The μSI-LOV analyzer has also been compared with other flow-through systems incorporating two-columns in tandem. Motomizu *et al.*[22] used anion and cation exchange resin disks for chromium speciation and preconcentration before ICP-AES determination. This method has a sample throughput comparable to our approach, but enrichment factors ≤10 were obtained for 5 mL sample volumes. Hashemi *et al.*[23] employed agarose-based chelating and anion exchange resins for automated sample treatment prior to off-line/on-line FAAS detection. However, sample volumes of around 80 mL were needed to attain an enrichment factor of merely 12, thus providing detection limits as high as 7.7 μg L^{-1} of chromium.

Validation

To evaluate the applicability and accuracy of the proposed analyzer for Cr(III)/Cr(VI) speciation, separation and preconcentration, a set of environmental waters as well as certified reference materials (*viz.*, SRM 1640-Natural Water and CRM-320 River Sediment) were analyzed for their Cr(III) and total chromium content, the concentration of Cr(VI) thus being calculated from the difference. Tap, river and lake water samples were spiked with two concentration levels of Cr(III) and Cr(VI) and the found values are given in Table 5. As can be seen, the recoveries of spiked samples range from 88 to 110%, thus implying the non-existence of multiplicative (non-spectral) matrix interferences.

For the river sediment material, there is no reason for determining Cr(VI) since this chemical form is converted into Cr(III) at the high temperature and highly acidic medium used for the wet digestion. A total chromium concentration of 127 ± 6 mg kg^{-1} was encountered which agreed well with the certified value (138 ± 7 mg kg^{-1}).

For the SRM 1640 water sample, the μSI-LOV speciation procedure gave concentrations of 32.2 ± 0.5 μg L^{-1} and 4.4 ± 0.4 μg L^{-1} for Cr(III) and Cr(VI), respectively. The total chromium concentration was in good agreement with the certified value (namely, 38.6 ± 1.6 μg L^{-1}). The large Cr(III)/Cr(VI) distribution ratio found for this particular sample is most likely due to the acidification of the reference material, which is known to accelerate the reduction of Cr(VI) in the presence of organic matter.[25,36,37]

The statistical comparison of means between the experimental and endorsed values for both SRM 1640 and CRM 320 using a significance *t*-test[38] revealed the non-existence of significant differences at a probability level of 0.05.

Table 5 On-line determination of trace levels of hexavalent and trivalent chromium in natural waters by hyphenation of μSI-BI-LOV with ETAAS

	Added/μg L^{-1}		Found/μg L^{-1} [b]		Recovery (%)	
Sample	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
Tap water[a]	—	—	0.027 ± 0.002	<LOD	—	—
	0.03	0.05	0.059 ± 0.001	0.048 ± 0.003	104	96
	0.08	0.06	0.108 ± 0.003	0.066 ± 0.004	100	110
River water[a]	—	—	0.019 ± 0.002	0.071 ± 0.002	—	—
	0.04	0.08	0.054 ± 0.002	0.16 ± 0.1	92	106
	0.1	0.1	0.118 ± 0.005	0.16 ± 0.1	99	94
Lake water	—	—	<LOD	<LOD	—	—
	0.04	0.08	0.035 ± 0.002	0.076 ± 0.004	88	95
	0.1	0.1	0.101 ± 0.005	0.104 ± 0.002	100	104

[a] Dilution factor, 1 : 10. [b] The results are given as the mean of 3 replicates ± SD.

Taking advantage of the great versatility of the hyphenated μSI-LOV, it can be anticipated that it will be possible to use it in combination with many other sample pretreatment strategies such as microwave digestion or ultrasound extraction in order to fulfill more on-line unit operations or even the use of more than one kind of sorbent, that is, bi-columns can be introduced into the LOV for speciation. Therefore, it is believed that a multitude of applications still need to be exploited in this field in the future.

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Paper IV

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Universal Approach for Selective Trace Metal Determinations via Sequential Injection−Bead Injection−Lab-on-Valve Using Renewable Hydrophobic Bead Surfaces as Reagent Carriers

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A new concept is presented for selective and sensitive determination of trace metals via electrothermal atomic absorption spectrometry based on the principle of bead injection (BI) with renewable reversed-phase surfaces in a sequential injection−lab-on-valve (SI-LOV) mode. The methodology involves the use of poly(styrene−divinylbenzene) beads containing pendant octadecyl moieties (C_{18}-PS/DVB), which are preimpregnated with a selective organic metal chelating agent prior to the automatic manipulation of the beads in the microbore conduits of the LOV unit. By adapting this approach, the immobilization of the most suitable chelating agent can be effected irrespective of the kinetics involved, optimal reaction conditions can be used for implementing the chelating reaction of the target metal analyte with the immobilized reagent, and an added degree of freedom is offered in selecting the most favorable elution mode in order to attain the highest sensitivity. The potential of the SI-BI-LOV scheme is demonstrated by taking Cr(VI) as a model analyte, using a 1,5-diphenylcarbazide (DPC)-loaded bead column as the active microzone. As this reaction requires the use of high acidity, it is also shown that the bead material exhibits excellent chemical stability at low pH values. On-line pH sample adjustment prevents alteration of the original distribution of chromium species while ensuring fast rates for the DPC−Cr(VI) reaction. The proposed procedure was successfully applied to the determination of trace levels of Cr(VI) in natural waters containing high levels of dissolved salts (such as seawater and hard tap water) without requiring any dilution step. Method validation was performed by determination of total chromium in an NIST standard reference material (NIST 1640, natural water) after Cr(III) oxidation, and the results were in good agreement with the certified value.

Although electrothermal atomic absorption spectrometry (ETAAS) is one of the most sensitive techniques for trace element determination, two challenges are still to be confronted: (i) the extremely low concentration of metal species in most environmental samples, which is often below the dynamic linear range of the detection instrument; and (ii) the severe spectroscopic or nonspectroscopic interferences caused by concomitant matrix components, particularly by high concentrations of electrolytes, that cannot be completely overcome by exploiting existing background correction devices.

Recent efforts have been directed to the design of reliable sample pretreatment methods for analyte isolation/preconcentration prior to sample presentation to the detector. Such pretreatment schemes are advantageously conducted in flow injection (FI) or sequential injection (SI) manifolds, where all unit operations can be effected in an enclosed and automated fashion and under strictly controlled chemical conditions.[1,2] Hence, a multitude of alternatives for on-line sample manipulation have been developed, including solvent extraction,[2−4] solvent extraction/back-extraction,[2,5] solid-phase microcolumn extraction involving ion-exchange, chelation, or hydrophobic interactions,[6−9] hydride and vapor generation,[10−13] precipitation/coprecipitation,[5,7,9] and sorption of neutral complexes in PTFE knotted reactors.[14,15]

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Schemes capitalizing on sorbent extraction prior to atomic spectrometric detection have attracted particular attention because of their simplicity and high separation and preconcentration capabilities. Yet, the long-term applicability of solid-phase reactors whenever employed in a permanently operational mode is frequently hindered by the following: (i) the progressive tighter packing of the material resulting in increase of back pressure; (ii) carryover effects; (iii) variations in sorbent volume; (iv) malfunctions of the active entities, including loss of functional groups, the latter being a common problem for reagent impregnated beads; (v) surface deactivation due to irreversible interfering species.

As recently reviewed,[16] the concept of renewable surfaces, so-called bead injection (BI), implemented in the third generation of flow injection analysis, i.e., SI−lab-on-valve (SI-LOV), is a promising microanalytical tool to alleviate the above-mentioned drawbacks and an excellent alternative to conventional solid-phase extraction for ultratrace metal determinations. However, two of the most stringent requirements to be satisfied by the sorbent material to be exploited in a renewable fashion are the bead size homogeneity and the spherical shape of all entities to prevent a compact settlement into the integrated microconduits. This explains the fact that most of the applications of SI-BI-LOV for the preconcentration of metal ions[2,16] or for the development of bioligand interaction assays[17,18] have employed hydrophilic beads (mainly ion exchangers or chelators) of the Sephadex type, which are perfectly spherical and uniform in size distribution.

From an analytical point of view, however, hydrophobic materials are more attractive than chelating or cation-exchange resins for assay of ultratrace metal through noncharged complex generation due to the materials' high tolerance to interfering ions and the presence of high salt content in the samples. A further advantage in selectivity can be gained via intelligent choice of the organic chelating agent. Although both poly(tetrafluoroethylene) and octadecyl-chemically modified silica gel beads possess superb analytical characteristics for metal preconcentration after formation of neutral complexes in terms of retention efficiency and enrichment factors, the physical features of these sorbents, such as density, bead shape, and particle distribution, make their manipulation in LOV troublesome.[19−21]

Ideally, the hydrophobic beads to be used should, beside exhibiting suitable morphology for being handled in the LOV assembly and being stable and unaffected by pH over a wide range, permit a suitable organic chelating reagent to be immobilized on the bead surface, independently of the kinetics involved here. This reagent should, in turn, be able selectively to form a stable complex with the target metal ion, which can be retained strongly onto the surface. By using an appropriate solvent, this one should then be capable of eluting reproducibly, and preferably quantitatively, the metal analyte, either as the metal ion itself (irrespective if this involves merely breaking the bonds between the metal ion and the chelating agent and even the binding to the support), or in the form of the entire generated complex, or possibly as a combination of both. If for some reason these schemes should fail, direct transportation of the analyte-loaded beads into the furnace should be regarded as an attractive option. Besides, when speciation procedures are to be accomplished, it should be possible to design on-line pH adjustment of the sample in order to prevent any change in the natural/original distribution of the metal species in question. All these very stringent requirements can only be met by using the so-called bead renewable concept, where the beads are renewed for each analytical cycle, since physically immobilized reagents cannot be relied upon to be employed repeatedly due to progressive leaching.

Thus, in this paper, a universal concept for selective and sensitive determination of trace metals exploiting hydrophobic surfaces in LOV is presented. The methodology is based on exploiting poly(styrene−divinylbenzene) beads containing pendant octadecyl moieties (C_{18}-PS/DVB) anchored at the polymer surface as renewable reagent carriers. To the best of our knowledge, this is the first work dealing with reagent-loaded C_{18}-PS/DVB beads in the LOV format prior to AAS measurements.

As opposed to C_{18}-silica materials, the polymeric entities are perfectly round-shaped, uniform in size, and resistant within the entire pH range, being thus well suited for chemical assays requiring acidic conditions. The analytical potential of this approach is demonstrated by the hyphenation of SIA-BI-LOV to ETAAS for chromium speciation analysis, using selective trace Cr(VI) determination. To this aim, the selective reagent 1,5-diphenylcarbazide (DPC), which reacts with Cr(VI) at high acidities, is physically immobilized onto the bead surfaces via $\pi-\pi$ stacking interactions between the aromatic rings of the reagent and the copolymeric matrix, as well as by partitioning into the alkyl chains, prior to manipulation into the miniaturized sample processing unit.

According to regulatory authorities, the concentration of Cr(VI) in environmental waters must be accurately determined as a consequence of its well-recognized toxicity. However, the low concentrations often found in natural waters make Cr(VI) virtually impossible to be determined directly by ETAAS.

EXPERIMENTAL SECTION

Instrumentation. A PerkinElmer AAnalyst 600 atomic absorption spectrometer furnished with a longitudinal Zeeman background corrector, an AS-800 autosampler, and a transversely heated graphite furnace equipped with pyrolytically coated graphite tubes was utilized as a detection instrument. The chromium hollow cathode lamp (Perkin-Elmer) was operated at a current of 25 mA, using a wavelength of 357.9 nm and a spectral band-pass of 0.7 nm. The time-resolved signals were processed in the integrated (peak area) mode.

The sequential injection system (FIAlab-3000, Bellevue, WA) consisted of two syringe pumps (Cavro, Sunnyvale, CA) of 10 (SP1) and 2.5 mL (SP2), containing the carrier solution and the pH adjustment solution, respectively; a peristaltic pump (PP), and a six-port multiposition valve (SV) mounted with the integrated LOV microbore unit. Each SP has a switching valve at its head,

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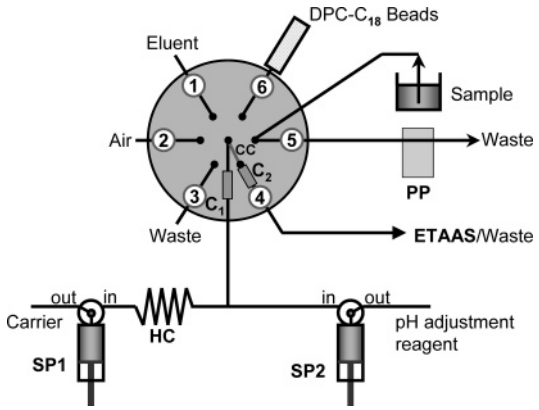

Figure 1. Schematic illustration of the SI-BI-LOV flow network handling DPC-loaded C_{18}-PS-DVB renewable surfaces for automatic preconcentration and determination of traces of Cr(VI) via ETAAS detection. Carrier, 0.5 mol L^{-1} HNO_3; pH adjustment reagent, 5 mol L^{-1} HNO_3; SP, syringe pump; PP, peristaltic pump; C_1 and C_2, LOV microcolumn positions; PP, peristaltic pump; HC, holding coil; CC, central communication channel; ETAAS, electrothermal atomic absorption spectrometry. DPC, 1,5-diphenylcarbazide.

which facilitates the communication of the syringe with either an external reservoir or the manifold. The miniaturized flow network is schematically illustrated in Figure 1. The manifold was built from PTFE tubing of 0.50-mm i.d. and 1.66-mm o.d, using a PEEK T-piece for connecting the conduits of both syringes to the selection valve. The holding coil (HC) was made from 1.32-mm-i.d., 1.93-mm-o.d. PTFE tubing, the length being 185 cm, corresponding to a volume of 2.5 mL. The delivery line to the atomizer was 106 cm long with a total capacity of 300 μL. This tube, which was manipulated by the ETAAS autosampler arm, was optionally used as a wasteline. The peristaltic pump was furnished with Tygon tubing of 1.22-mm i.d. and 2.80-mm o.d., allowing for a flow rate of 2.1 mL min^{-1}. The main goal of this liquid driver is to rinse the sampling conduit (viz., the common port 5 of the LOV) between standards/samples of different concentrations, thereby minimizing risks of sample carryover. On changing the solution to be analyzed, the PP is engaged to aspirate solution past the common port, whereupon it is stopped, and the sample plug is aspirated by SP1 into the HC.

The LOV microconduit is fabricated from hard PVC and contains six microchannels (1.66-mm i.d. and 12.0-mm length), the peripheral ports of which (1−6) can be made to address the central port of the LOV via the central communication channel (CC) in the SV. Two of these channels (the central one and the one to port 4) serve as microcolumn positions for the reagent-carrying beads. To contain the beads within the cavities of the LOV module and prevent them from escaping, the outlets of the columns are provided with small pieces of rigid PEEK tubing of 1.60-mm o.d. (Upchurch Scientific, Oak Harbor, WA) working as stoppers. The diameter of the rods is slightly smaller than that of the LOV conduits, thereby allowing liquid to flow freely along the walls, but effectively entrapping the sorptive hydrophobic beads.

The operational sequences of the SI-LOV system and ETAAS were fully automated and synchronized. Two independent computers were used to communicate with the FIAlab-3000 setup and the AAnalyst 600 detector, respectively, yet, all unit operations were readily controlled by the FI/SI computer through an intelligent electronic interface, as described elsewhere.[22]

Reagents, Solutions, and Sorbent Preparation. All chemicals were of analytical-reagent grade, and doubly deionized water (18.2 MΩ cm) obtained from a Millipore system (Milllipore Synthesis A10, France) was used throughout for solution preparation. The carrier consisted of 0.5 mol L^{-1} HNO_3, while on-line pH adjustment was achieved by pumping a 5 mol L^{-1} HNO_3 solution by means of the external syringe pump SP2. A 90% (v/v) methanol/water solution was employed as eluent. Working standard solutions of Cr(VI) were obtained by appropriate dilution of a 1000 mg L^{-1} stock solution of K_2CrO_4 (Merck) in water.

The hydrophobic reagent carriers consisted of octadecyl chemically modified poly(styrene−divinylbenzene) beads (C_{18}-PS/DVB; Polysorb MP-1, Transgenomic Inc., Omaha, NE), with a mean particle size of 40 μm. Prior to reagent immobilization, a 1:20 (w/v) bead suspension was prepared in methanol and filtered through a glass filter (15−40 μm) to remove particles smaller than 40 μm that cannot be quantitatively entrapped within the LOV microcolumn cavities. The bead material retained on the filter was cleansed with two 5-mL portions of methanol and finally vacuum-dried for 5 min. To prepare the active surfaces for Cr(VI), 1.0 mL of a 3.6% (m/v) DPC solution in methanol was added to 0.2 g of precleansed beads. A final concentration of 5% (v/v) methanol was ensured by adding 250 μL of methanol before bringing the suspension volume to 25 mL with water. The resulting suspension was preserved from light and subjected to continuous stirring for 30 min. The adsorption process of the reagent onto the bead surfaces can be followed by the naked eye because of the developing characteristic dark pink of the DPC-modified sorbent. The sorptive material was aspirated into a 1-mL plastic syringe, which then was mounted vertically on port 6 of the integrated microsystem. After settlement on the bottom of the reservoir, the DPC-loaded beads were readily handled via SP1 into the various positions of the LOV unit. The impregnated beads can be used for more than 2 days provided that they are stored refrigerated at 4 °C whenever not in use.

All glassware was rinsed prior to use with a 25% (v/v) concentrated nitric acid solution utilizing a washing machine (Miehle, model G 7735 MCU) and afterward cleansed with Milli-Q water.

Operational Procedure. The SI-BI-LOV method for handling of the reagent-loaded C_{18}-PS/DVB beads involves five different operational stages, namely, system preconditioning, analyte sorption onto the modified hydrophobic entities, removal of matrix constituents, analyte elution, and bead disposal. The operational details of a complete measuring cycle including flow rates and volumes handled, selected ports of the SV, and positions of SP valves, are compiled in Table 1, and summarized as follows:

System Preconditioning (Step 1). Initially, the HC is washed with 2500 μL of carrier, and the 8-cm line connecting SP2 with the T-connector is filled with the pH-adjustment acid solution. After these preliminary operations, SP1 is set to aspirate consecutively carrier solution from the external reservoir and methanol solution from port 1. Thereafter, the solvent segment plus 300 μL of carrier are, via the communication channel, directed to port 4 for rinsing column positions C1 and C2 and the connecting line to the ETAAS

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Table 1. Operational Sequence for SI-BI-LOV-ETAAS Determination of Cr(VI) Using DPC-Loaded C_{18}-PS/DVB Beads

step	SP1 valve[a]	SP2 valve[a]	SP1	SP2	LOV position	flow rate (μL s^{-1})	volume (μL)	comment
1								system preconditioning
	out	out	aspirate		1	100	1050	(a) filling of SP1 with carrier
	in	out	aspirate		1	50	400	(b) solvent aspiration
	in	out	dispense		4	50	700	(c) washing of ETAAS line
2								sample and bead loading and preconcentration
	in	out	aspirate		5	100	2000	(a) loading of HC with sample
	out	out		aspirate	5	50	500	(b) filling of SP2 with pH adjustment solution
	in	out	aspirate		2	50	75	(c) introduction of air segment into HC
	in	out	aspirate		6	4	25	(d) aspiration of bead suspension
	in	in	dispense	dispense	4	60 (SP1)/ 15 (SP2)	2100 (SP1)/ 500 (SP2)	(e) analyte preconcentration
3								sample cleanup
	in	out	dispense		4	60	500	(a) rinsing of sorbent bed
4								filling of ETAAS line with air
	in	out	aspirate		2	50	430	(a) introduction of an air zone into HC
	in	out	dispense		4	30	380	(b) filling of ETAAS line with air
5								activation of ETAAS
6								elution of sorbed species
	in	out	aspirate		1	10	30	(a) eluent aspiration
	in	out	dispense		4	4	30	(b) elution of analyte loaded beads
	in	out	aspirate		2	50	380	(c) aspiration of second air segment (during this sequence the eluent is stopped in C2)
	in	out	dispense		4	10	430	(d) delivery of eluate into ETAAS furnace via air-segmentation
7								bead discarding
	in	out	aspirate		1	100	300	(a) solvent aspiration
	in	out	dispense		4	80	300	(b) dispensing of methanol plug to C2 microcolumn
	in	out	aspirate		1	100	300	(c) transfer of bead suspension from C2 to C1 positions
	in	out	dispense		3	50	550	(d) removal of beads

[a] The position "out" means connection of SP with the external reservoir; "in" means connection of SP with the manifold.

detector. Thus, a 750-μL carrier volume is left in SP1 for subsequent use, i.e., sample cleanup and bead removal (re below).

Sample and Bead Loading and Analyte Preconcentration (Step 2). First, the required volumes of sample and pH adjustment solutions are drawn from their respective containers. A small air segment is then aspirated into HC to place the head of the sample plug just exactly behind the T-confluence for appropriate subsequent on-line merging of sample with acid solution. A metered portion of the DPC-coated beads is next aspirated slowly into microcolumn C1. The communication channel is then connected to the peripheral port 4, and SP1 and SP2 are activated simultaneously. As a result, Cr(VI) reacts with the immobilized DPC in an acid environment. During the solid-phase derivatization reaction and preconcentration of the target analyte, the beads in microcolumn C1 are concomitantly transferred to the C2 position.

Sample Cleanup (Step 3). For the removal of weakly or nonretained matrix constituents from the hydrophobic material, an amount of 500 μL of the previously stored carrier solution is propelled to port 4 to cleanse both the analyte-containing microcolumn and the ETAAS line after sample loading.

Elution (Steps 4−6). To fulfill the accommodation volume requirements of the graphite tube and to preserve the identity of the eluate zone, the air-segmented elution approach was selected for transportation of the preconcentrated analyte into the ETAAS instrument. To this end, a gentle stream of aspirated air is initially used to replace the carrier solution in the ETAAS line. The ETAAS temperature program, which was adapted from the manufacturer's recommendations (see Table 2), is activated at this instant. Subsequently, SP1 is set to aspirate 30 μL of the methanol solution, which then is dispensed forward to slowly strip out the metal chelate and DPC reagent from microcolumn C2. The analyte-enriched zone is finally propelled by a second air segment into the graphite furnace via the autosampler tip.

Table 2. Temperature Program for the ETAAS Determination of Chromium

step	temperature (°C)	ramp time (s)	holding time (s)	argon flow rate (mL min^{-1})
drying 1	110	5	20	250
drying 2	140	5	40	250
pyrolysis	1500	10	20	250
atomization	2300	0	4	0
cleansing	2450	1	3	250

Bead Discarding (Step 7). Once the autosampler tip has been moved out of the furnace, the uncoated C_{18}-PS/DVB beads are discarded by transferring them back to microcolumn C1 as a sorbent−methanol suspension and afterward delivered to waste through port 3.

The ETAAS program is synchronized with the SI-BI-LOV protocol, whereby the next sample starts to be processed automatically in the flow network while the former one is being pyrolyzed and atomized in the furnace.

RESULTS AND DISCUSSION

Performance of the SI-BI-LOV Scheme with Hydrophobic Beads. One of the most common approaches for flow-through solid-phase extraction of target metal ions via hydrophobic interactions on reversed-phase materials is the on-line formation of neutral chelates in a coiled reactor incorporated into the flow network, the chelate subsequently being retained onto a sorbent-packed permanent column.[7,23,24] An alternative is to introduce the selective functional chelating group onto the sorbent matrix by means of either chemical or physical binding. The preimpregnation of the bead material with a solution containing the chelating ligand should, however, be regarded as the most promising strategy to be tailored to SI-LOV schemes with renewable surfaces, since it directly overcomes the most severe drawback of physical immobilization when exploited in a permanent fashion, i.e., the short lifetime of the loaded sorbent, because of the gradual flushing out of the reagent during sample percolation or analyte elution.[25]

In addition, in our model application using the easy-to-handle C_{18}-PS/DVB beads, it was proven that the reaction product from Cr(VI) and DPC was not appreciably retained in the LOV-packed microcolumn whenever generated in an on-line sequential injection mode. This result is in agreement with earlier observations that revealed low retention efficiencies for moderately nonpolar metal chelates on C_{18}-PS/DVB reactors.[20] This is probably due to the relatively slow kinetics of adsorption of the resulting chelate on the surface of the copolymeric beads. Furthermore, it should be borne in mind that the derivatization reaction for Cr(VI) with DPC in a strongly acid medium is reported to constitute a two-step process involving the oxidation of DPC (H_4L) to diphenylcarbazone (H_2L) by the target analyte via formation of a metastable metal−reagent intermediate with a special sterical conformation, and finally the chelation of the oxidized reagent with the generated Cr(III) species according to the following reaction:[26]

$$2CrO_4^{2-} + 3H_4L + 8H^+ \rightarrow [Cr(HL)_2]^+ + Cr^{3+} + H_2L + 8H_2O$$

Since the increase of polarity of the formed chelate also is hindering the on-line sorption of the target metal on the renewable hydrophobic surfaces, the physical binding approach for the neutral DPC reagent in an off-line mode was found most appropriate for the LOV procedures. Not the least this is because the immobilization time can be selected at will aiming at ensuring an adequate sorption of the organic chelator onto the sorbent. Preconcentration of the analyte on the reagent-coated bead microcolumns, where the reaction is driven by an excess of solid reagent, also compensates for both the miniature dimensions of the LOV reactors and the inherent dynamic nature of the on-line retention process.

Though the reaction between Cr(VI) and DPC develops fast merely at high concentrations of mineral acids, sample acidification is reported to bias the analytical results in natural waters due to the progressive reduction of the hexavalent chromium to Cr(III) by dissolved organic matter.[27,28] To operate at the experimental conditions of maximum sensitivity without shifting the natural equilibrium between oxidation states, a hybrid FI−SI manifold was assembled, involving the incorporation of an external syringe pump for acid delivery. The purpose of this configuration is to obtain full benefit of the ruggedness of the liquid driver and the precise timing control of SI systems. Thus, the untreated sample zone is made to merge synchronously with a defined volume of acid solution delivered via the ancillary syringe pump like flow injection networks. Hence, automated pH adjustment of the sample plug is attained immediately prior to its reaching the DPC-coated beads, as loaded in the LOV unit, entailing minimum alteration of the original distribution of species in the sample.

Investigation of Chemical and Physical Parameters. The preparation of the DPC-carrying particles prior to the SI-LOV sample treatment was found to be crucial for successful performance of the automated approach in terms of retention capability for Cr(VI) and manipulation of the sorptive surfaces. The effect of the DPC concentration on the analytical features of the physically modified beads was evaluated from 0.0072 to 0.144% (v/v). In all cases, the suspensions contained 5% (v/v) methanol to prevent excessive floating of the hydrophobic beads while ensuring adequate adsorption of the organic derivatizing compound. Experimental results revealed that concentrations below 0.036% DPC (v/v) rendered lower sorption efficiencies owing to insufficient reagent immobilization, as detected visually by the pale pink color of the resulting active surfaces. Though the analytical sensitivity remained almost constant from 0.036% (v/v) and onward, the highest concentration of DPC used (i.e., 0.144%) was selected for further investigations in view of the higher tolerance to interfering species (e.g., oxidizing agents), and also because of the slight increase of the size of the entities (nominal bead diameter of 40 μm) that facilitates their manipulation within the LOV conduits and their entrapment as microcolumn reactors onto the PEEK stoppers.

It is well known that the reaction of DPC with Cr(VI) is favored at high sample acidities.[26] Preliminary investigations using strong acids of different natures (viz., HCl, HNO_3, and H_2SO_4) at a concentration level of 0.5 mol L^{-1} H^+ revealed that an oxidizing acid is mandatory for reaction development, thereby explaining the widespread acceptance of the latter two chemicals for selective Cr(VI) determinations.[29,30,31] The effect of the concentration of nitric acid on the integrated absorbance at a sample/acid ratio of 4 is illustrated in Figure 2. The enrichment of the LOV-packed beads decreases sharply for concentrations of ≤0.5 mol L^{-1} H^+, which is in agreement with previous observations.[30] Thus, automatic pH adjustment with a 5 mol L^{-1} HNO_3 solution that yields a medium acidity suitable for the solid-phase derivatization reaction (i.e., 1.0 mol L^{-1} H^+) was selected for the remainder of the explorations.

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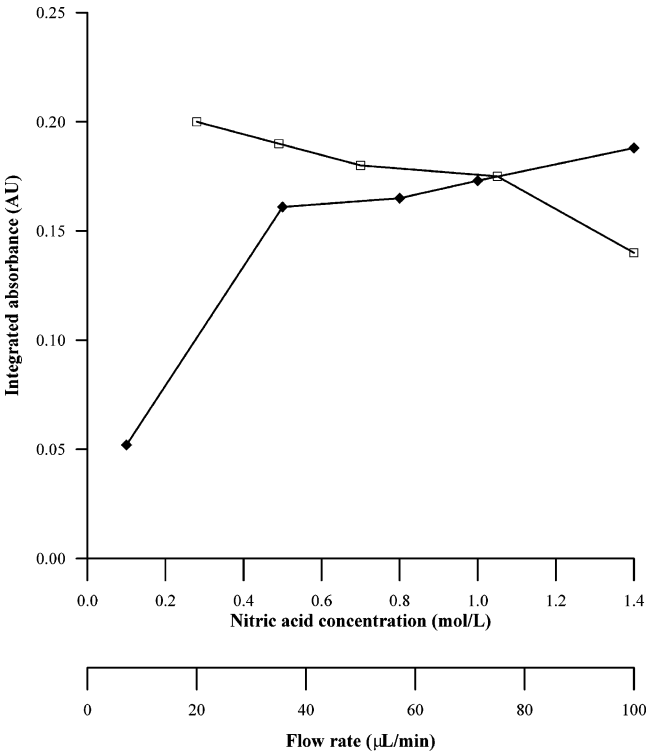

Figure 2. Influence of the acidity of the reaction medium (◆) and the sample loading flow rate (□) on the analytical signal. Loading volume, 2.0 mL; sample to nitric acid volume ratio, 4; eluent volume, 30 μL; standard concentration, 0.67 μg L^{-1} Cr(VI).

To investigate the potential use of the packed-bead column as a stationary reactor for consecutive assays, attempts to elute the complexed chromium species with the impregnated reagent were performed using increasing concentrations of nitric acid. It was proven that eluent concentrations as high as 5 mol L^{-1} HNO_3 release less than 30% of the retained analyte. As a consequence of the irreversible sorption, quantitative recovery of the sorbed metal ion was effected using organic solutions, e.g., 90% (v/v) MeOH, aimed at stripping the immobilized Cr−H_2L chelate. Concomitant removal of the immobilized DPC takes place, thereby precluding the sorbent loaded in the LOV to be reutilized. Yet, the bead injection renewable scheme should be regarded as a unique approach for handling single-use reactors, which are automatically discarded after each analytical cycle.

Regarding eluent volume, experimental results confirmed that the chromium chelate was effectively stripped from the C_{18}-PS/DVB beads by using 30 μL of 90% MeOH in an air-segmented mode, implying a 25% lower volume than that required in previous sorptive extraction methods involving permanent packed-bed microcolumns.[32] Thus, both the restricted volumetric requirements of the graphite platform and the reliable accommodation of the organic eluate in the atomizer tube, without excessive longitudinal distribution of the liquid, were fulfilled. The elution step was performed at a low flow rate (viz., 4 μL s^{-1}) to prevent buildup of back pressure, which would result in an irregular and partial introduction of the analyte-enriched zone into the ETAAS detector. In addition, sufficient intimate contact time between the eluent and the sorbent material (~15 s) is guaranteed in the optimized procedure. Actually, the discontinuous SI system might, if called for, even feature straightforward implementation of additional stopped-flow strategies to increase this contact time.

The influence of the total flow rate (sample + acid) on the preconcentration behavior of hexavalent chromium onto the reactive surfaces was evaluated from 20 to 100 μL s^{-1}, as shown in Figure 2. A slight decrease of both sensitivity and repeatability was observed only at flow rates higher than 80 μL s^{-1}. Since the miniaturized, noncompressible, hydrophobic reactor allows rather high rates without deterioration of the analytical performance of the sorptive process, a total flow rate of 75 μL s^{-1} was selected for the sorption step as a suitable compromise between sorption efficiency and desirable sample throughput, the rate of which is limited by decreasing the flow rate. In fact, the LOV microcolumns tolerate higher loading flow rates than those applicable in the jet-ring cell BI configurations for appropriate sensitivity.[30] It should also be stressed that the selected preconcentration flow rate is not admissible in SI-BI-LOV schemes involving hydrophilic ion exchangers of the Sephadex type, as the functionalized beads become squeezed above 20 μL s^{-1} and therefore they cannot be effectively entrapped on top of the small PEEK rods.[19]

Comparison of the Analytical Features of the SI-BI-LOV System with DPC-Loaded C_{18}-PS/DVB Beads with Those of Permanently Packed-Bead Microcolumns and Knotted Reactors. Under the optimized chemical and physical variables detailed in the foregoing section, the figures of merit of the SI-LOV system for Cr(VI) determination are summarized in Table 3, including statistical parameters, sampling throughput, microcolumn dimensions, sample volume, loading rate, sorption efficiency, enrichment factor, and concentration efficiency. The detection and determination limits are calculated as the concentration of analyte providing an integrated absorbance signal equivalent to 3 and 10 times the standard deviation of the blank, respectively. The reproducibility is expressed as the precision obtained by six consecutive measurements of a 0.3 μg L^{-1} Cr(VI) standard solution using the renewable loaded beads. The enrichment factor is calculated as the ratio of the linear range sensitivity of the proposed preconcentration method and that obtained by direct ETAAS injection of 30 μL of standard solutions. The retention efficiency is defined as the ratio between the retained amount of analyte and the maximum available for DPC according to the reaction stoichiometry. The value given in parentheses corresponds to the sorption yield calculated by comparison of the analytical signal obtained following Cr(VI) preconcentration with that measured by direct injection into the ETAAS of the total amount of loaded analyte. The concentration efficiency is defined as the product of retention efficiency and sample throughput per minute, thus dictating the enrichment factor achieved by the preconcentration system per minute.

The analytical performance of the LOV assembly using reagent-supporting hydrophobic entities has been critically compared with recently reported on-line preconcentration methods for Cr(VI) determination via ETAAS, based either on sorbent extraction using a permanent microcolumn placed at the outlet tip of the autosampler arm[32] or on the molecular sorption of the neutral reaction products of Cr(VI) with ammonium pyrrolidine dithiocarbamate onto the inner walls of PTFE knotted reactors.[33]

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Table 3. Comparison of the Analytical Performance of the SI-BI-LOV System Using DPC-Loaded C_{18}-PS/DVB Beads with That of Open Tubular PTFE Knotted Reactors and Permanent Sorbent Microcolumns Prior to Cr(VI) Determination via ETAAS

	preconcentration unit		
parameter	SI-LOV with renewable DPC-loaded C_{18}-PS/DVB beads	column-in-tip[32]	knotted PTFE reactor[33]
regression equation (Cr, μg L^{-1})	0.2691 [Cr] + 0.0022	0.089 [Cr] + 0.034	0.3669 [Cr] + 0.0096
correlation coefficient	0.9982	0.997	0.9992
linear range (μg L^{-1})	0.12−1.5	0.4−8	0.01−1.25
reactor volume (μL)	15	35	245
column dimension (length (cm) × i.d. (cm))	0.7 × 0.166	0.5 × 0.3	125 × 0.05
aspect ratio (length/diameter)	4.2	1.7	
sample volume (mL)	2	2	5
loading flow rate (mL min^{-1})	4.5	2	5
sample frequency (h^{-1})	15	28	16.7
retention efficiency (%)	38.8 (19.4)	11.2	13.1
enrichment factor	12.9	5.6	16.3
concentration efficiency (min^{-1})	3.2	2.6	4.5
detection limit (μg L^{-1}) (3σ)	0.03	0.08	0.016
determination limit (μg L^{-1}) (10σ)	0.12	0.4	nr[a]
precision (%)	3.8	3.5	2.4

[a] nr, not reported.

Despite the larger capacity of the microcartridge attached to the ETAAS autosampler arm for sorptive materials, the retention efficiency, enrichment factor, and detection and determination limits for Cr(VI) attained with the transient LOV microcolumns were more than 2-fold improved as compared with the column-in-tip mode, as deduced from data compiled in Table 3. This is attributed to the different nature of both packing materials and the better hydrodynamic design of the LOV open column reactors. In fact, the aspect ratio[8]—defined as the ratio between the length and diameter of the column—which is regarded as a core factor influencing the performance of flow-through sorptive preconcentration systems, is 2.5-fold better in the proposed fully automated assembly. Not the least comparable coefficients of variation were attained for both conceptually different procedures, regardless of the continuous manipulation of the reactive surfaces in the LOV microconduits, probably as a consequence of the absence of compaction or clogging problems whenever using the easy-to-handle hydrophobic renewable beads.

Flow-through preconcentration of noncharged organometallic chelates onto the inner walls of knotted reactors has been reported to be a promising alternative to traditional on-line extraction systems using sorbent columns.[14,15] The lower hydrodynamic impedance of the open tubular reactor permits the use of higher sample loading rates, thus yielding higher enrichment factors for identical sample throughput. However, according to the results presented in Table 3, the SI-BI-LOV system allows loading flow rates comparable to those applied to knotted reactors because of the minute dimensions of the LOV-packed column and the renewable nature of the reagent carriers. Even though the inner surface of the PTFE open tubular reactor is slightly larger than the effective surface area of the LOV beads, and the loading sample volume applied is 2.5-fold higher, the enrichment factor of the SI-LOV procedure with coated C_{18}-PS/DVB beads is close to that of the PTFE reactor because of the better retention efficiencies of the packed materials.

Interferences. As opposed to ion-exchange resins, hydrophobic sorbent materials feature improved tolerance to high salt content samples, whereby their implementation in flow-through systems for on-line sample treatment prior to ETAAS has recently attracted particular attention.[7,24] Not the least because one can by intelligent selection of the derivatization or complex-forming reagent obtain increased selectivity. DPC was considered as the most appropriate reagent for sorbent loading in our application, since it is well documented to be practically selective for Cr(VI); only Mo(VI) has been reported to yield a similar two-step oxidation/complexation reaction.[34,35] The potential interfering effect of Cd and Cu ions by chelate formation with DPC (or its oxidized form, diphenylcarbazone)[36] was also evaluated, using standard solutions containing 0.5 μg L^{-1} Cr(VI) together with metal concentrations at different levels. Ratios of interfering species to analyte of >1000 (higher concentrations not tested) for Mo(VI), Cd, and Cu were tolerated at the 10% interference level under the optimized SI-BI-LOV conditions with no need for masking agents. It should be stressed that the tolerated ratio for metal ions, such as Cu, is more than 5-fold better than that reported for on-line preconcentration schemes with sorbent-in-tip cartridges[32] or with PTFE knotted reactors using ammonium pyrrolidine dithiocarbamate as a derivatization reagent.[33] This result reveals the low Cu-catalyzed oxidation rates of DPC at the high acidities applied for the Cr(VI) reaction.[36] The negligible effect of high concentrations of Mo(VI) is attributed to the excess of immobilized DPC and the large aspect ratio of the microcolumn design. In fact, the retention efficiency for Cr(VI) was proven unaffected by the concomitant presence of oxidizing reagents, such as $KMnO_4$, at a concentration level of 5×10^{-5} mol L^{-1}, the latter reagent actually being used later in connection with validation purposes (see below).

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Table 4. Determination of Trace Levels of Hexavalent Chromium in Environmental Waters by On-Line Hyphenation of SI-BI-LOV Using DPC-Impregnated C_{18}-PS/DVB Entities with ETAAS

sample	added (μg L^{-1})	found (μg L^{-1})[b]	recovery (%)
natural water		<LOD	
(SRM 1640)[a]	0.22	0.21 ± 0.01	95.4
	0.45	0.47 ± 0.02	104.4
	0.67	0.69 ± 0.02	103.0
tap water		0.040 ± 0.002	
	0.22	0.25 ± 0.02	96.2
	0.45	0.50 ± 0.04	102.0
	0.67	0.74 ± 0.04	104.2
seawater		0.108 ± 0.009	
	0.22	0.33 ± 0.02	100.6
	0.36	0.45 ± 0.03	96.2
	0.49	0.63 ± 0.04	105.3

[a] Certified concentration of total chromium, 38.6 ± 1.6μg L^{-1}. Dilution factor, 1:100. [b] Results are expressed as the mean of 3 replicates ± standard deviation.

In contrast to previously reported speciation methods tolerating Cr(III)/Cr(VI) ratios of ≤2,[32,37] synergistic effects become appreciable in our system handling solid DPC reagent only at a 15-fold higher concentration of Cr(III) for a 0.5 μg L^{-1} Cr(VI) level. Yet, it should noted that sample carryover was detected whenever concentrations of Cr(III) at the high-microgram per liter level were injected into the flow network.

Application of the SI-BI-LOV Procedure. The on-line bead injection preconcentration system was applied to the determination of Cr(VI) in different types of environmental waters including tap water and seawater samples. After collection, samples were filtered by using a 0.45-μm-pore size membrane and analyzed without delay. Whenever stored, the filtered samples were not subjected to any additional treatment, such as acidification, to prevent modification of the original distribution of the oxidation states. The application of the method of standard additions to the whole set of analyzed samples revealed the absence of multiplicative (nonspectroscopic) matrix interferences, as deduced from the recovery values detailed in Table 4.

The accuracy of the SI-LOV approach with DPC-loaded C_{18}-PS/DVB beads was assessed using an appropriate dilution of the standard reference material SRM 1640 (trace elements in natural water), for which the total chromium content is certified (namely, 38.6 ± 1.6 μg L^{-1}). According to the results presented in Table 4, no content of Cr(VI) was found in the SRM 1640, which is attributed to the reduction to Cr(III) in the presence of natural organic matter under the acidic conditions of the reference material that has been stabilized with nitric acid at a concentration of 0.5 mol L^{-1}. Therefore, several oxidizing agents, already exploited for total chromium determination, such as ammonium cerium(IV) sulfate, hydrogen peroxide in NaOH, ammonium peroxodisulfate, and acidic potassium permanganate, were tested for batchwise Cr(III) oxidation. The first two reagents were not appropriate for the on-line preconcentration system owing to the high blank signals recorded and the generation of vapor bubbles in the flow network, respectively. A modification of the off-line $KMnO_4$ oxidation procedure recommended by Tunçeli and Rehber-Türker[38] was finally adopted. Despite raising the reaction temperature from 45 to 80 °C and increasing the reaction time from 15 to 30 min for a concentration of 5×10^{-5} mol L^{-1} $KMnO_4$, quantitative Cr(III) conversion was not obtained for this particular matrix, as detected by Cr(III) spikes. The application of a 3-level standard addition method rendered a total concentration of 39.9 ± 1.6 μg L^{-1} Cr expressed as the 95% confidence limit for the extrapolated *x*-value,[39] which is in good agreement with the certified NIST value.

CONCLUSION

In this paper, reagent-supporting C_{18}-PS/DVB beads have been exploited in the bead injection fashion in the SI-LOV mode for on-line sample treatment and trace metal preconcentration prior to ETAAS measurements. With the precoating approach adopted, there are no limiting requirements for the adsorption of the organic compounds used, provided that appropriate hydrophibicity is ensured. By proper intelligent selection of the chelating agent, improved selectivity for the target metal ions is attained as compared with the ion exchangers conventionally used in SI-LOV systems. Bead injection protocols are well suited for accommodating physical immobilization procedures for reagent loading because of the renewable nature of the sorptive entities, thereby eliminating the problems due to leakage of the active derivatization compounds weakly bound to the hydrophobic material, as encountered in flow-through permanent packed-bed reactors or solid-state-based optical sensors whenever intended for long-term continuous monitoring purposes. In addition, off-line reagent immobilization approaches for C_{18}-PS/DVB particles offer enhanced versatility as the kinetics of the adsorption of a given chelating agent on the surface of the hydrophobic matrix can be accounted for.

The SI-BI-LOV concept should be regarded as a unique strategy to implement reagent-based solid-phase extraction assays with no need for full reversibility of the sorption/elution process, as demonstrated in this work via trace level determination of Cr(VI). The inability to desorb quantitatively the generated Cr(III) ions from the sorbent, even at high concentrations of mineral acids, was overcome by dissolving the metal chelate and immobilized DPC with a nonaqueous solvent and discarding the unloaded beads after each analytical sequence. Moreover, this scheme prevents inactivation of the packed reactor due to the progressive oxidation of the DPC by the analyte itself or by dissolved oxygen or potentially oxidizing species present in the analyzed samples. Preservation of the original distribution of oxidation states in environmental waters was accomplished via assemblement of a hybrid FI−SI setup for on-line adjustment of acid concentration.

Current research is being focused in our group to expand this novel concept for automatic handling of hydrophobic sorbents in a renewable means to other chemistries with final aims of improved selectivity and sensitivity in comparison with traditional wet chemical assays. Special interest is also being paid to the development of speciation analysis based on bead injection exploiting SI-LOV as a versatile front end to ETAAS.

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Paper V

On-line dynamic extraction and automated determination of readily bioavailable hexavalent chromium in solid substrates using micro-sequential injection bead-injection lab-on-valve hyphenated with electrothermal atomic absorption spectrometry

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A novel and miniaturized micro-sequential injection bead-injection lab-on-valve (μSI-BI-LOV) fractionation system was developed for on-line microcolumn soil extraction under simulated environmental scenarios and accurate monitoring of the content of easily mobilisable hexavalent chromium in soil environments at the sub-low parts-per-million level. The flow system integrates dynamic leaching of hexavalent chromium using deionized water as recommended by the German Standard DIN 38414-S4 method; on-line pH adjustment of the extract by a 0.01 mol L^{-1} Tris–HNO_3 buffer solution; isolation of the chromate leached from the matrix constituents onto a Q Sepharose strong anion-exchanger freshly packed into the microconduits of the μSI-assembly; air-segmented elution of the sorbed species by a 40 μL plug of 0.5 mol L^{-1} NH_4NO_3 (pH 8) eluent; and detection by electrothermal atomic absorption spectrometry (ETAAS). The effect of simulated acidic rain on the accessibility of chromate forms for plant uptake was also investigated. The proposed approach offers several advantages over conventional speciation/fractionation protocols in the batch mode, including immediate separation with concomitant preconcentration of the released chromate, minimization of Cr(VI) to Cr(III) interconversion risks, enhanced accuracy, and non-existence of re-adsorption/re-distribution problems along with a detailed pattern of the kinetics of the leaching process. The reliability of the proposed method was evaluated *via* spiking of a moderately polluted agricultural soil material (San Joaquin Soil-Baseline Trace Element Concentrations) with water-soluble Cr(VI) salts at different concentration levels. The potential of the μSI-BI-LOV set-up with renewable surfaces for flame-AAS determination of high levels of readily bioavailable chromate in contaminated soils is also addressed.

Introduction

Speciation analysis of physicochemical forms of chromium is currently a topic of major interest as a consequence of the increased anthropogenic levels of chromium in the environment due to industrial discharges—including metal electroplating, leather tanning, chromate ore processing, spray painting and wood treatment[1]—and the well-known different ecological significance of the two most relevant valence states, *i.e.*, trivalent and hexavalent chromium.[2,3] Trivalent chromium is an essential micronutrient in the diet of mammals to maintain effective glucose, lipid and protein metabolism.[2,4] In contrast, hexavalent chromium is highly toxic and carcinogenic for a variety of organisms as a result of its elevated oxidation potential and the ability to penetrate biological membranes.[2,5,6] In addition, epidemiological studies have shown that high exposures to Cr(VI) in the workplace cause dermal sensitisation and human respiratory diseases.[7,8] Both chromium species also differ significantly with respect to environmental mobility in solid substrates, hexavalent chromium salts of alkaline metals being those most soluble in neutral and slightly acidic media.[1,9] All these considerations make detection and quantification of hexavalent chromium rather than the total metal content a subject of major concern for risk assessment in ecology, water pollution and environmental management.

Monitoring of pollutants in environmental solids is usually accomplished *via* extraction/digestion methods followed by chemical analysis of the extracts/digests. Extraction methods intended for speciation/fractionation analysis are based on exposing the solid sample to an extracting reagent able to dissolve the targeted compounds or pre-defined physicochemical phases of ecological interest.[10] In fact, fractionation methods have gained widespread acceptance in environmental studies because they can reveal relevant information regarding pollutant soil-phase associations as well as elucidating the mode of occurrence, magnitude of available reservoirs, and potential migration of elements in natural environments.[11]

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Metal fractionation protocols are traditionally conceived as operationally defined single or sequential extraction methods performed under a pseudo-equilibrium regime.[12–15] Yet, recent trends have been directed at designing flow-through multiple-step dynamic fractionation procedures mostly involving microcolumn extractions aimed at imitating field conditions more correctly than their batch counterparts.[16] Automated flow-systems with on-line detection have, however, been used solely for fractionation of elements in highly contaminated solid substrates, the raw extracts generated being in all instances delivered directly to the hyphenated analytical instrument,[17–21] mostly ICP-MS,[17,18,20] without any prior sample treatment step. Hence, their applicability to highly salted matrices or to extracts containing ultra-trace metal contents is rather limited. Actually, these methods fail to monitor the most ecotoxicological significant fractions of trace metals, *i.e.*, the water-soluble or exchangeable pools, which determine the readily available, and thus toxic, forms for biota uptake.

Amongst the various extraction approaches for determining chromium in soils and sediments[22,23] the most commonly used protocol for quantitation of Cr(VI) involves alkaline digestion at a pH around 12 as endorsed by the USEPA, also known as method 3060A,[24] followed by the spectrophotometric analysis of the digests *via* the diphenylcarbazide (DPC) method. The main pitfalls of the standard method are related to its operation under unrealistic environmental scenarios, mainly to minimize undesired interconversion between oxidation states,[22,25] its low sensitivity for trace analysis,[25] and the solubilisation of humic matter which makes the subsequent analysis of Cr(VI) by DPC questionable.[26] In addition, the application of external energy sources such as ultrasonication,[27,28] focused microwaves,[29] and magnetic stirring or plate heating[22,24] are aimed at releasing not only soluble but also sparingly soluble and partially insoluble Cr(VI).[30] Therefore, these procedures lack the ability to ascertain the potentially harmful ecological and human health effects caused by the access of soluble soil-borne hexavalent chromium to groundwaters or plant uptake *via* surface runoff or irrigation waters.

In this paper, an automated and rugged micro-sequential injection lab-on-valve (μSI-LOV) microcolumn fractionation system hyphenated to ETAAS detection and integrating on-line matrix separation and additional analyte preconcentration is proposed for the first time for expeditious and accurate determination of the content of readily mobilisable forms of Cr(VI) in solid substrates of environmental origin utilizing distilled water, as recommended by the DIN 38414-S4 method,[31] and artificial acid rain as well. The third generation of flow injection analysis, *viz*. μSI-LOV, particularly in the bead-injection (BI) fashion with renewable sorptive entities, has shown significant advantages for on-line handling and pre-treatment of liquid samples of relative complexity as regards to trace metal separation, concentration and automated quantification as recently reviewed.[32,33] Yet, to the best of our knowledge the μSI-BI-LOV concept has not been exploited as an analytical tool for accommodating flow-through dynamic speciation/fractionation schemes of solid samples so far. The discontinuous flow nature of SI-LOV makes the hyphenation with discrete non-continuous detectors, such as ETAAS, uncomplicated, thus yielding improved sensitivity, as compared with the classical DPC spectrophotometric method, for reliable monitoring of ultratrace amounts of readily-leachable hexavalent chromium.

Experimental

Instrumentation

An atomic absorption spectrometer (PerkinElmer AAnalyst 600) with a Zeeman background correction, and a transversely heated graphite furnace equipped with pyrolytically coated graphite tubes was used for the determination of chromium. A wavelength of 357.9 nm with a spectral bandpass of 0.7 nm and an operating current of 25 mA were set for the chromium hollow cathode lamp (Perkin Elmer). The temperature program for the chromium analysis is shown in Table 1. The manufacturer's recommendations have been slightly modified for facilitating the progressive volatilisation of the eluate solvent. The signals were recorded in the integrated (peak area) mode.

The FIAlab-3000 sequential injection system (Bellevue, WA) was equipped with the integrated LOV central sample-processing unit mounted atop a six-port selection valve (SV), two high-precision bi-directional syringe pumps (SP1 and SP2) with a capacity of 10 mL and 2.5 mL, respectively, and a peristaltic pump. A diagram of the whole system is shown in Fig. 1. The LOV microbore assembly (diameter, 5 cm; thickness, 1 cm) made from hard PVC contains a central port which can communicate with the other six micro channels (1.66 mm i.d./12.0 mm length) through the central communication conduit (CC) in the SV. The microchannel connecting SP with CC and that of port 4 serve as containers for bead-microcolumns C_1 and C_2 in which PEEK stoppers (Upchurch Scientific, Oak Harbor, WA), which have a dimension slightly smaller than that of the channel, are used for retaining the beads while allowing the solution to flow freely. The soil column, bead container (syringe) and eluent solution were attached to the remaining peripheral ports of the LOV. The holding coil (HC) was connected with the central port and CC *via* microcolumn C_1. The two-way valves at the heads of SP1 and SP2 facilitate the communication of each syringe with either an external reservoir (carrier or buffer) or with the central port in the LOV manifold *via* a PEEK T-connector. The manifold was built from PTFE tubing of 0.50 mm i.d./1.66 mm o.d., except the 170 cm long HC which was made from PTFE tubing of 1.50 mm i.d./2.10 mm o.d. corresponding to a volume of 3.0 mL. The delivery line to the atomizer consists of a 0.6 mm i.d. tube with a total length of 106 cm. This tube, which is manipulated by the ETAAS autosampler arm, was optionally used as a waste line.

Table 1 Temperature program for the ETAAS determination of chromium in soil extracts following on-line pre-treatment

Step	Temperature/°C	Ramp time/s	Holding time/s	Argon flow rate/mL min^{-1}
Drying 1	110	5	35	250
Drying 2	130	5	45	250
Pyrolysis	1500	25	20	250
Atomization	2300	0	4	0
Cleansing	2450	1	3	250

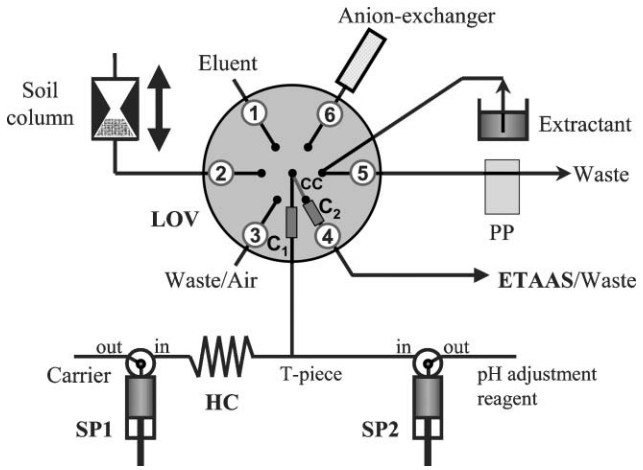

Fig. 1 Schematic diagram of the μSI-BI-LOV-ETAAS system for dynamic fractionation of Cr(VI) in environmental solids. Carrier, 0.01 mol L^{-1} Tris–HNO_3 buffer at pH 8.0; on-line pH adjustment reagent: 0.02 mol L^{-1} Tris–HNO_3 buffer at pH 8.0; eluent, 0.5 mol L^{-1} NH_4NO_3–NH_4OH at pH 8.0; anion-exchanger, Q Sepharose; SP1/SP2, syringe pumps 1 and 2; C_1 and C_2, LOV microcolumn positions; HC, holding coil; CC, central communication channel; PP, peristaltic pump, ETAAS, electrothermal atomic absorption spectrometer.

The flow-through port of the LOV (port 5) is utilized as the inlet for the leaching solution into the flow network, the outgoing channel being connected to a peristaltic pump (operated at 2.0 mL min^{-1}), thereby permitting thorough washing of the conduits between different extractants. The LOV port at position 3 plays a double role, serving both as outlet for final bead disposal and as an inlet for air (*i.e.*, for air-segmentation purposes).

The specially designed chemical-resistant PEEK extraction microcolumn has been described in detail elsewhere.[34] Briefly, it contains a central bi-conical shaped sample container (as shown in Fig. 1) and it is furnished with filters, filter supports and caps at both ends. The formerly used PTFE membrane filters with 1.0 μm pore size were here replaced with 0.45 μm cellulose acetate filters (Minisartfilters, Sartoriuos, Göttinger) for efficient retention of particulate matter within the sample holder.

The operational procedures of the μSI-BI-LOV system were computer controlled by the associated FIAlab software and synchronised with the commands for the activation of the ETAAS program through an intelligent electronic interface.[35]

Reagents, solutions and sample

All reagents were at least of analytical grade. Doubly de-ionised water (18.2 MΩ cm) obtained from a Milli-Q system (Millipore Synthesis A10, France) was used throughout. All flasks and beakers for solution preparation were cleaned with a 25% (v/v) concentrated nitric acid solution followed by repeated washing with Milli-Q water.

A 0.1 mol L^{-1} Tris-buffer stock solution was prepared by dissolving 12.114 g of Tris(hydroxymethyl)-aminomethane in 1000 mL water which was afterward adjusted to pH 8.0 by dropwise addition of 1.0 mol L^{-1} HNO_3. It was further diluted to 0.01 mol L^{-1} and 0.02 mol L^{-1} to serve as carrier and on-line pH adjustment solutions, respectively. The eluent consisted of a 0.5 mol L^{-1} NH_4NO_3–NH_4OH buffer at pH 8.0. Double deionised water and simulated acid rain at pH 3.5 (adjusted with diluted nitric acid) were selected as mild extractants for determination of readily bioavailable Cr(VI). For calibration purposes, working standard solutions of hexavalent chromium were obtained by step-wise dilution of a 1000 mg L^{-1} stock solution of K_2CrO_4 in water.

A commercially available strong anion exchanger Q Sepharose™ Fast Flow (Amersham Biosciences, Sweden), as contained in 20% ethanol solution and with a mean particle size of 90 μm, was used directly in the LOV with no need for any additional swelling protocol. The beads were contained in a dedicated syringe reservoir mounted atop of port 6.

A moderately-polluted agricultural soil (San Joaquin Soil-SRM 2709, Baseline Trace Element Concentrations) purchased from the National Institute of Standards and Technology (NIST) was used for evaluation of the performance of the on-line soil fractionation/solid-phase extraction system. The standard material, which was highly homogenized during the NIST preparation, contained a total chromium concentration of 130 ± 4 μg g^{-1}. The bi-conical microcolumn was packed with soil amounts ranging from 20 to 100 mg as detailed under Results and Discussion.

Operating procedures

The complete operational sequence for Cr(VI) fractionation in soil with further on-line analysis of the extracts using the μSI-BI-LOV scheme is listed in Table 2, and summarized as follows:

1. System preconditioning and bead loading. Firstly, SP1 and SP2 are filled with carrier and pH adjustment solution, respectively. Thereafter, the CC is connected to port 3 to aspirate air into HC, thereby leaving the channel conduit filled with air for subsequent use. A metered volume of ion-exchanger is next aspirated slowly (*viz.*, 0.3 mL min^{-1}) into microcolumn C_1, and transferred to C_2 by 400 μL eluent as described in Table 2.

2. Soil extraction. SP1 is set to consecutively aspirate a minute air plug and a 500 μL extracting reagent portion. By reversing the flow, the leaching solution is introduced into the soil reactor for effecting the dissolution of weakly bound chromate. Collection of soil extract plus an additional 200 μL air zone back into HC is realized by reverse motion of SP1 at a relatively slow rate.

3. Isolation of Cr(VI) from matrix ingredients. For optimal pH adjustment of the extract solution with the buffer provided by SP2, the surplus of air aspirated into HC is delivered to port 3. SP1 and SP2 are then activated simultaneously to propel the soil extract and the buffer solution to port 4 for loading of the ion-exchange resin with Cr(VI). A clean-up step for removal of weakly or non-retained matrix constituents from the LOV conduits is also carried out.

Table 2 Operating procedure of the μSI-BI-LOV-ETAAS system for on-line fractionation of Cr(VI) in soil

Sequence and description	SP1 valve[a]	SP2 valve[a]	SP1	SP2	LOV position	Flow rate/μL s^{-1}	Volume/μL
1. System preconditioning and bead loading							
(a) Aspiration of carrier and buffer	Out	Out	Aspirate	Aspirate	—	100 (SP1) 50 (SP2)	1230 (SP1) 600 (SP2)
(b) Rinsing of port 3	In	Out	Dispense	—	3	50	400
(c) Filling port 3 tubing with air	In	Out	Aspirate	—	3	100	200
(d) Aspiration of eluent into HC	In	Out	Aspirate	—	1	50	400
(e) Collection of beads into C_1	In	Out	Aspirate	—	6	5	25
(f) Moving beads to C_2 and cleansing with eluent	In	Out	Dispense	—	4	10	400
(g) Flushing beads and ETAAS line with carrier	In	Out	Dispense	Dispense	4	50 (SP1) 20 (SP2)	425 (SP1) 50 (SP2)
2. Soil extraction							
(a) Air segment aspiration	In	In	Aspirate	—	3	50	70
(b) Loading of extracting reagent into HC	In	In	Aspirate	—	5	50	500
(c) Mixing of extractant with packed soil	In	In	Dispense	—	2	50	570
(d) Collection of soil extract	In	In	Aspirate	—	2	5	770
3. Cr(VI) separation							
(a) Moving of the head of extractant to T-connector	In	In	Dispense	—	3	50	200
(b) On-line buffering and Cr(VI) separation/preconcentration	In	In	Dispense	Dispense	4	50 (SP1) 50 (SP2)	610 (SP1) 550 (SP2)
(c) Rinsing of sorbent microcolumn with carrier	In	In	Dispense	—	4	50	250
4. Elution and measurement							
(a) Introduction of air into HC	In	In	Aspirate	—	3	50	750
(b) Filling of ETAAS line with air	In	In	Dispense	—	4	20	380
(c) Aspiration of eluent	In	In	Aspirate	—	1	10	40
(d) Elution of analyte loaded beads	In	In	Dispense	—	4	10	50
(e) Stopped flow (delay 7 s)							
(f) Activation of ETAAS program: tip of autosampler arm moves into the graphite tube							
(g) Transportation of eluate into the atomizer	In	In	Dispense	—	4	10	360
(h) Autosampler arm moves back to the original position, ETAAS runs the temperature program							
5. Bead disposal							
(a) Dispensing of carrier to C_2	In	In	Dispense	—	4	50	200
(b) Bead transportation from C_2 to C_1 positions	In	In	Aspirate	—	4	100	200
(c) Withdrawal of used beads	In	In	Dispense	Dispense	3	50	300

[a] The position "out" means connection of SP with the external reservoir, while "in" means connection of SP with the manifold.

4. Elution and measurement. Before elution, the remaining solution in the ETAAS line is replaced by an air segment. Then, 40 μL of eluent are aspirated into HC, and subsequently directed in an air-sandwiched format to column C_2 wherein it remains for 7 s (stopped-flow). The ETAAS program is, at this moment, automatically activated and the autosampler tip moves into the dosing hole of the graphite tube. The eluate is finally propelled by SP1 into the graphite tube for Cr(VI) determination.

5. Bead disposal. After measuring the content of Cr(VI) in the extract, the anion-exchange beads are discarded by transferring them back to C_1 and afterward delivered to waste through port 3.

The ETAAS program is synchronized with the LOV method, whereby the next extraction comprising the multiple-step fractionation protocol starts to be effected while the former extract is being pyrolyzed and atomized in the furnace.

Results and discussion

Investigation of chemical variables and operating parameters in the μSI-BI-LOV for separation and preconcentration of Cr(VI) species

In order to find the best operational and chemical conditions for separation, preconcentration and speciation analysis of ultra-trace levels of chromium in the μSI-BI-LOV flow system prior to ETAAS quantitation, a series of preliminary investigations was conducted using aqueous solutions (attached to port 2) in lieu of soil extracts. Amongst the various parameters affecting the performance of the sorbent bead-injection preconcentration in terms of sorption efficiency for Cr(VI), the bead material, sample acidity, loading flow rate, eluent type and stripping-out conditions and tolerance to potential interfering species are regarded as the most crucial ones.

Selection of sorptive material for Cr(VI) preconcentration/separation

Bearing in mind the inability of the atomic absorption spectrometer for discriminating chromium species and its low tolerance to high concentrations of electrolytes, the target analyte should be isolated from trivalent chromium and other matrix ingredients prior to presentation to the detector. This is accomplished in this work by exploiting anion-exchangers as a sorptive medium for Cr(VI) taking into account its anionic nature in most natural environments.

Among the various sorbents exploited in a permanent fashion for Cr(VI) enrichment, namely Dowex 1-X8[27–29] and Sepharose/Sephadex-type[36] exchangers, the latter ones are preferable for handling in the microbore LOV unit as

renewable surfaces. The reasons that make them ideal for manipulation in the third generation of flow injection analysis without risks of bead settlement in the integrated conduits mainly lie in their hydrophilic nature, perfectly spherical shape and narrow size distribution. Additional features of functionalised polysaccharide-type solid phases for implementation as temporary reactors in flow systems include high binding capacities, excellent flow properties, and high chemical and physical stabilities.

Initially, a strong anionic exchanger (QAE Sephadex), wherein the diethyl-(2-hydroxypropyl)aminoethyl moiety is chemically attached to a cross-linked dextran matrix was evaluated. This sorbent, being supplied as a dry powder, requires a swelling pre-conditioning protocol with a high electrolyte concentration (*e.g.*, 2 mol L^{-1} $NaNO_3$) prior to moistening with buffer solution at the application pH. Although QAE-Sephadex showed appropriate adsorption performance for Cr(VI) traces, its utilization as a microcolumn in LOV cavities is limited by the volume changes of the resin-bed as a consequence of sorbent shrinking/swelling upon application of solutions of different composition and/or ionic strength. Thus, a cross-linked 6% agarose furnished with diethyl-(2-hydroxypropyl)aminoethyl, so-named Q Sepharose Fast Flow, which is physically more resistant than Sephadex exchangers and can be directly manipulated in the flow network with no need for any ancillary treatment, was selected for Cr(VI) enrichment in the LOV.

Sample loading pH

For optimum retention of hexavalent chromium onto the packed-bead Q Sephadex microcolumn, the acid–base nature of the target metal species should be taken into consideration. Anionic forms are the prevalent species at pH >2.8, which explains the fact that no significant differences on the efficiency of the sorbent reactor for collection of Cr(VI) in standard solutions were observed within the pH range 3.5–8.5 (higher values not tested), which is in accordance with earlier observations made by Hashemi *et al.*[36] using batchwise column preconcentration systems. Yet, the tolerance to potentially interfering monovalent anions in real-life samples was improved at pH $\geqslant$ 8.0 as a consequence of the stronger affinity of the resin for the predominant divalent chromate oxoanion. Moreover, slightly alkaline media are commonly recommended for stabilization of Cr(VI) solutions (see below).[22,28] Buffering of the standards was accomplished by addition of Tris–HNO_3 buffer (pH 8.0). The dependence of the buffer concentration on the analyte recovery was studied from 5×10^{-3} to 0.1 mol L^{-1}. Significant chromate breakthrough (>20%) was detected for buffer concentrations above 0.05 mol L^{-1} Tris as a result of the pre-elution effect occasioned by the surplus of nitrate. A 0.01 mol L^{-1} Tris–HNO_3 buffer solution was selected for the remaining studies as a compromise between Tris buffer capacity and retention efficiency for Cr(VI).

Sample loading flow rate

As opposed to Sephadex-type exchangers,[37] highly cross-linked Sepharose beads are able to endure high solution flow rates with negligible squeezing,[38] whereby the transient sorptive entities are effectively trapped within the LOV microchannels with no possibility for escaping through the space between the PEEK stopper and the wall of the LOV unit. High loading rates are particularly desirable for achieving elevated concentration factors whenever trace elements are determined. The effect of loading flow rate on the preconcentration behaviour of Cr(VI) onto the ion-exchanger was investigated from 25 to 125 μL s^{-1}. The analytical readouts revealed excellent repeatabilities and comparable sensitivity in the whole range of applied rates for Q Sepharose. In fact, flow rates as high as 100 μL s^{-1} are tolerated in the LOV-BI sorption mode because of the minor deterioration of the analytical sensitivity ($\leqslant$3%) as compared with the lowest loading rate assayed, *i.e.*, 25 μL s^{-1}.

Elution procedure

Elution of retained species from packed-bead ion-exchange columns is frequently performed by a sharpened increase of the ionic strength of the mobile phase. Yet, one of the fundamental requirements for appropriate quantification of the released species is the compatibility of the elution medium with the detection instrument. It is well recognized that atomic spectrometers such as ETAAS lack sufficient tolerance to directly analyze solutions of high salt content. Thus, for example, the analysis of chromium species in 0.5 mol L^{-1} NaCl has been reported to be cumbersome because of the severe background absorption or interferences occurring in the presence of high concentrations of chloride.[39] Recovery of chromate and suitable quantification by means of ETAAS is also feasible *via* application of a pH gradient through the anion-exchanger.[40] A concentration of 2 mol L^{-1} HNO_3 was hence utilized as eluting solution to decrease the affinity of the target species for the sorptive material. However, a 20% signal reduction was detected by direct injection of standards prepared under these acidic eluting conditions as compared with those in Milli-Q water as a result of the contribution of non-spectral interferences for the selected ETAAS operational sequence. Improvement of the analytical performance in terms of elution yields and minimization of interfering effects was accomplished by exploiting an electrolyte buffer (NH_4NO_3–NH_4OH) adjusted to the same alkaline condition as that of the carrier medium (*i.e.*, pH 8.0), but at a higher ionic strength, which is in accordance with previous observations.[28] In fact, according to the electrochemical reduction potential of the Cr(VI)/Cr(III) redox pair, stabilization of Cr(VI) occurs in bases, thus minimizing the possible oxidation of the organic groups of the matrix beads, which has been described for co-polymer type ion-exchange resins.[41] In order to prevent the existence of a pH gradient during the elution/preconditioning/preconcentration steps that might induce the generation of Cr(III) ions by oxidation of the organic beads—that would be then unavailable for the resin moieties—alkaline media were chosen for the overall analytical protocol.

To fulfil the restricted volumetric requirements of the graphite platform of the atomizer and the reliable accommodation of the eluate in the tube, discrete eluent volumes $\leqslant$50 μL should be utilized. However, in the proposed system,

an air-segmented 50 μL plug of 0.8 mol L^{-1} buffer delivered at 10 μL s^{-1} into the loaded microcolumn rendered incomplete Cr(VI) stripping with carryover of 3%, as detected by a multiple elution protocol. This drawback was overcome by the precise fluidic control and flow programming of SI systems that foster the straightforward implementation of stopped-flow approaches. Actually, by halting a mere 40 μL of NH_4NO_3–NH_4OH buffer within the LOV cavity containing the Cr(VI)-loaded beads for 7 s the carryover was reduced to 0.75%. Thus, these elution conditions were adopted for further investigations.

The concentration of the alkaline buffer proved to be critical for suitable signal-to-noise ratios. Quantitative recovery of sorbed species was obtained in a single eluent plug by buffer concentrations $\geqslant$0.5 mol L^{-1} NH_4NO_3–NH_4OH whenever a stopped-flow strategy was executed, yet the electrolyte content of the eluate should be as low as possible for reliable ETAAS quantification, although one can take advantage of the fact that most of the salts are vaporized during the pyrolysis step. Therefore, a 0.5 mol L^{-1} NH_4NO_3–NH_4OH buffer (pH = 8) was selected for the remainder of the optimization and fractionation protocols.

Under the optimized chemical and physical variables detailed above, the retention efficiency of the anion exchanger for hexavalent chromium, defined as the ratio of the retained amount of analyte onto the microcolumn to the total amount available in the sample, was as high as 90 $\pm$ 3%, serving as a superb solid-phase reactor for our purpose of speciation/separation/preconcentration of traces of soluble Cr(VI) in soil samples.

On-line dynamic fractionation schemes for trace levels of Cr(VI) in environmentally relevant solid samples

The open architecture of the central processing unit in a μSI-LOV analyzer mounted atop a multi-position selection valve allows random access to not only reagent reservoirs and liquid solutions, but offers the possibility of hyphenation to ancillary modules at peripheral ports for facilitating unit operations on-line. This is here demonstrated by the inclusion of an external soil-containing microcolumn.

Therefore, dynamic soil extraction processes as occurring *in natura* can be simulated *via* multiple leaching schemes involving steady renewal of eluent solutions that capitalize on the application of discontinuous forward–backward flow as precisely coordinated and controlled by the syringe pump.

Configuration of the μSI-LOV-BI set-up for on-line soil extraction

As opposed to previous works dealing with on-line FI/SI microcolumn fractionation of environmental solids,[17–21,34] the specially designed soil container is attached to one of the peripheral ports of the LOV in lieu of being implemented into the manifold, with the upper outlet open to the atmosphere. The upright disposition is intended to withhold the entire substrate in the lower conical cavity of the column and facilitate the stripping out of the extractant moistening the packed solid. During each single extraction step of the overall operational protocol, the extracting reagent was pumped forward into the soil column at a flow rate of 50 μL s^{-1} to ensure fluidized-bed like conditions for improved mixing between leachant and sample, while the extract was progressively pulled inward at a slower rate, namely, 7 μL s^{-1}, for a more realistic simulation of water percolation through environmental soil bodies. The continuous on-line renewal of the eluent in intimate contact with the soil material prevents the problem of metal re-adsorption in freshly exposed surfaces as detected in the most labile fractions (*i.e.*, water-soluble, exchangeable, and acid soluble) of batchwise sequential extraction procedures for trace metals.[12,42] In addition, by application of a bi-directional flow, back-pressure or clogging effects due to soil compaction that are frequently observed in continuous-flow or uni-directional flow injection fractionation manifolds[16] are not encountered in the μSI-LOV manifold.

The microflow SI-arrangement can, in fact, be viewed as an FI–SI hybrid system due to the external syringe pump (SP2) assembled for on-line pH adjustment. The role of this ancillary liquid driver is not only to minimize competitive sorption of interfering anions for solid-phase extraction of Cr(VI), but also to prevent undesirable Cr(VI) to Cr(III) interconversion under the slight acidic medium of the aqueous extractants. Actually, batchwise extraction methods for Cr(VI) using distilled water or acidic reagents are prone to render biased results as a consequence of the accelerated reduction of the target analyte by dissolved organic matter and other reductants in the time span from digestion/extraction to analysis.[22,43] This can be solved elegantly in our system by the combined action of immediate pH adjustment of the extract to alkaline conditions (pH 8.0) and the subsequent isolation/preconcentration of the hexavalent chromium from matrix ingredients on the anion-exchanger. Since the soil extract and buffer are mixed at a 1 : 1 ratio, a concentration of 0.02 mol L^{-1} Tris–HNO_3 buffer solution was employed in SP2 according to the results presented above.

In addition, the irreversible accumulation of matrix components on the sorbent material as detected by the progressive darkening of the bead surfaces when used repeatedly was fully circumvented by exploitation of solid-phase extraction in a bead-injection renewable fashion, that is, the active microcolumn is discarded after each extract analysis and replaced by a fresh portion of ion-exchange resin.

Effect of coexisting ions

The tolerance of the solid-phase preconcentration method to potentially interfering species either in the sorption process or in the final determination by ETAAS was ascertained using a fixed concentration of 0.2 μg L^{-1} Cr(VI) standard solution and variable amounts of foreign species. To this end, the influence of the prevailing water soluble anionic species in soil extracts, such as Cl^-, HCO_3^-, NO_3^-, SO_4^{2-} and the most ubiquitous cationic species, such as Ca^{2+} and Mg^{2+}, which might lead to non-spectroscopic interferences during analysis, was evaluated. A given concentration level of a chemical species was regarded as interferent whenever the analytical readout of the Cr(VI) standard was affected by more than 10%. The tolerated interferent/analyte ratios of anions prepared from the corresponding sodium salts are listed in Table 3. As can be seen, the

Table 3 Tolerance of the μSI-LOV-BI system to concomitant anions present in soil extracts[a]

Foreign species	Tolerated interferent/Cr(VI) ratio
Cl^-	5×10^6
HCO_3^-	1×10^7
NO_3^-	5×10^6
SO_4^{2-}	5×10^4

[a] Concentration of Cr(VI) = 0.2 μg L^{-1}.

ion-exchanger materials can endure rather high concentrations of monovalent anions such as Cl^-, HCO_3^- and NO_3^-, which is in accordance with earlier observations.[28]

As for the potential interfering effect of Ca^{2+} and Mg^{2+} on the detection instrument, it should be borne in mind that cationic species are not sorbed on the anionic-exchanger so that concentrations as high as 200 mg L^{-1} are admissible for accurate dynamic fractionation of Cr(VI). Higher concentrations could not be tested as a consequence of the competitive sorption of concomitant nitrate on the active sites of the agarose resin.

In order to investigate the effect of organic matter and soil matrix ingredients released during water extraction on the ETAAS measurements of Cr(VI) at the low μg L^{-1} level, the batchwise DIN-38414-S4 protocol was applied to the agricultural San Joaquin NIST soil. The application of a 3-level standard addition method to the filtered extract using the LOV system rendered comparable sensitivity to that of the external calibration, thus revealing the inexistence of multiplicative matrix interferences, which is the result of the on-line sample clean-up step effected *via* bead-injection analysis.

Application and validation

The performance of the proposed on-line LOV-BI microcolumn fractionation method was evaluated by using a NIST agricultural soil (San Joaquin SRM 2709) as a model of environmental solid moderately polluted with trace elements. The 100 mg solid sample contained within the conical microcolumn was continuously exposed to air-sandwiched 500 μL distilled water plugs in a single forward–reversed motion. The implementation of the air-segmentation approach was aimed at monitoring the location of the discrete extractant zone through the sample line and preventing undue dispersion of the extract volume into the carrier stream prior to isolation of Cr(VI) onto the ion-exchanger. A 1 : 5 soil to extractant volume was adopted for each leaching step rather than the 1 : 10 ratio of the DIN protocol in order to obtain a more detailed pattern, *i.e.*, higher resolution, of the partitioning process without excessive dilution of the mobilised Cr(VI) in the extractant medium. The multiple-step dynamic extraction profile, so-called extractogram, which yields a thorough insight into the leaching kinetics of the targeted metal fraction under environmentally simulated water infiltration/percolation conditions, is shown in Fig. 2. As can be seen, the water-soluble forms of hexavalent chromium are promptly and quantitatively stripped from soil compartments in less than 8 fractions ($\leqslant$4.0 mL), and therefore they should be accurately determined for reliable risk assessment of chromium pollution in soil as a consequence of the immediate accessibility for

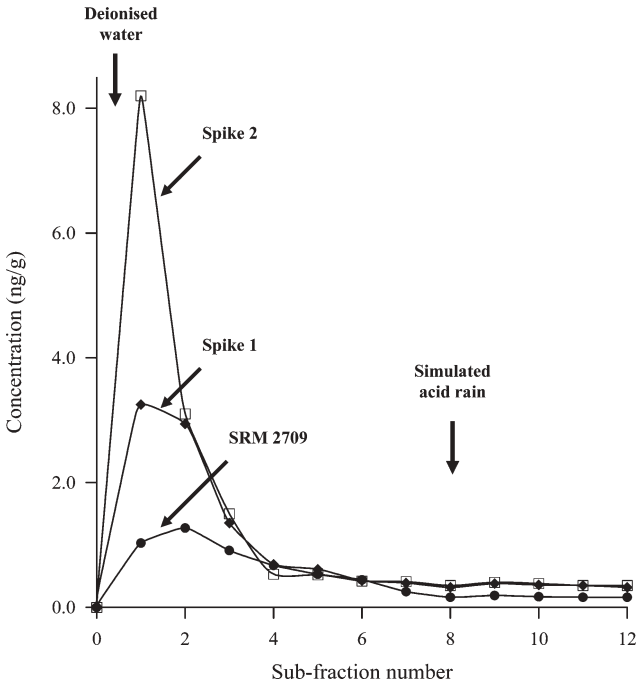

Fig. 2 Extraction profiles of readily bioavailable Cr(VI) in SRM 2709 and spiked samples as obtained from the μSI-LOV microcolumn system using mild extractants. Soil amount, 100 mg; sub-fraction volume, 500 μL; spike 1, 5.0 ng g^{-1}; spike 2, 8.0 ng g^{-1}.

fauna and flora uptake. Furthermore, it should be borne in mind that the proposed on-line LOV-ETAAS hyphenated system with integrated chromate preconcentration circumvents the lack of sensitivity of the traditional DPC photometric method for quantification of the most ecotoxicological significant forms of chromium. The effect of increased acidity (acid rain) on the extractability of chromate was mimicked by replacing distilled water with a diluted nitric acid solution at pH 3.5, since artificial rainwater at this pH has been previously utilized for batch column leaching experiments.[44] No appreciable leachability increase upon acidification was observed whenever both extractants were applied sequentially, as shown in Fig. 2. This result reveals the efficiency of distilled water for quantitative removal of soluble (surface bound) chromate in an on-line dynamic mode, and the ability of the soil material to raise the pH of the applied extractant, thus precluding the additional release of sparingly-soluble forms of Cr(VI).

Quantification of extractable chromium in the various fractions was performed by using an external calibration procedure against matched standards under the very same operational conditions as for the dynamic fractionation analysis, except that the soil containing microcolumn was replaced by a 2.5 m long open-tubular reactor (1.0 mm i.d., 2.0 mm o.d.) and the extractant at port 5 by the standard solutions. A straightforward calibration protocol based on using different volumes ($\leqslant$1800 μL) of a single Cr(VI) standard was selected, because preliminary tests demonstrated the compatibility of mass calibration with the sorptive sample treatment. The mass calibration curve was linear from 0.02 to 0.6 ng Cr(VI) and fitted the equation $Y = 0.6631X + 0.0112$ ($r = 0.9992$) where Y and X stand for the integrated

Table 4 Water extractable concentrations of hexavalent chromium for SRM 2709 and different soil spikes at variable concentration levels using the μSI-BI-LOV microcolumn set-up

Soil sample	Soil amount/mg	Concentration added/ng g^{-1}	Concentration found/ng g^{-1}	Recovery (%)	t_{exp}
SRM 2709	100	—	4.9 ± 0.3	—	—
Spike 1	100	5.0	9.5 ± 0.5	96 ± 5	1.39
Spike 2	100	8.0	13.9 ± 0.6	108 ± 4	2.89
Spike 3	20	40	44 ± 3	98 ± 6	0.52
Spike 4	20	55	64 ± 2	107 ± 3	3.55

[a] Results are expressed as the mean of 3 extraction replicates ± SD

absorbance and injected amount of Cr(VI), respectively. For the microcolumn soil extraction, although some bubbles were formed eventually at the inlet of the column due to the decreased pressure during the backward flow of extractant, both the on-line separation step and the application of a mass-based least squares regression circumvented any interfering effect due to gases generated in the flow manifold. In contrast, bubble formation represents one of the most severe constraints of the DPC-photometric assay for Cr(VI) determination following on-line extraction, as recognized by Grate and Taylor.[43]

The water extractable content of Cr(VI) in the moderately polluted San Joaquin Soil obtained as a summation of the various extracts composing the multiple-step fractionation procedure amounted to 4.9 ± 0.3 ng Cr(VI) g^{-1} soil, which represents less than 5×10^{-3}% of the certified value of total chromium in the sample. This is attributed to the high reduction potential of this agricultural soil due to the organic matter content. In fact, the batchwise EDTA-extractable chromate for this SRM standard is reported to be merely 0.1% of the total chromium in the sample.[39] Yet, it must be borne in mind that EDTA is capable of dissolving soluble, sparingly-soluble and insoluble forms of hexavalent chromium by chelation of the counterions in insoluble salts (*e.g.*, lead and barium chromates), thereby overestimating the pool size of readily available forms of Cr(VI).

For validation of the dynamic μSI-LOV fractionation system, strict comparison with the manual DIN38414-S4 standard method was not feasible because of the different operationally defined conditions and the influence of re-distribution phenomena in the equilibrium-based procedure. Besides, no certified solid material for readily bioavailable hexavalent chromium is currently commercially available. Reliability and ruggedness of the analytical method was evaluated *via* Cr(VI) spikes. Earlier researchers[39,45] recommended effecting the spikes directly on the extracts rather than on the solid substrates due to soil redox reactions and immobilization processes that often occasion low or near-zero recoveries in batchwise analysis. However, fortification of the SRM substrate with soluble salts of Cr(VI) (*viz*., potassium chromate) did not pose any problem in the developed system whenever analyses were conducted without delay as a result of the drastic reduction of extraction time as compared with conventional end-over-end methods. Experimental results obtained by spiking variable amounts of soil with Cr(VI) levels ranging from 5 to 55 μg kg^{-1} are compiled in Table 4. Application of a second-order polynomial regression equation was needed for quantitation of the water-soluble Cr(VI) content in the first extract fraction of the spike of highest concentration. A statistical *t*-test[46] was used for each spike to ascertain whether or not there was a significant difference between the concentration of Cr(VI) added and that found. Since the overall experimental values of $|t|$ are below the critical value at the 0.05 significance level, *i.e.*, 4.30, no significant differences were encountered for any set of data, thus indicating the nonexistence of multiplicative (non-spectroscopic) matrix interferences. For this particular soil, there is then no need to utilize the standard addition method that, whenever applied to flow-through fractionation schemes, not only demands highly repeatable extractograms but also implies tedious and time-consuming operational procedures as the sample containing microcolumn must be replaced for each addition.[19] Reproducibility of the overall fractionation/solid-phase preconcentration method and soil homogeneity were assessed from data presented in Table 4. Maximum relative standard deviations of 5.3% and 6.8% were found for 100 and 20 mg soil packed columns, respectively. Yet, for handling poorly homogeneous soils, larger substrate amounts might be accommodated in the custom-built column to guarantee sample representativeness, as recently demonstrated.[21,47]

The potential extension of the developed analyzer for fractionation of Cr(VI) in highly contaminated soils by on-line hyphenation with FAAS rather than ETAAS has also been investigated. To this end, an additional injection valve was implemented as an interface between the discontinuous μSI-LOV flow approach and the continuously operating detector for injection of the extracts into the FAAS nebuliser stream.[21] Analyte breakthrough—calculated from the residual concentration of Cr(VI) during loading of the anion exchanger—did not occur up to 180 ng Cr(VI), corresponding to a minimum concentration of 9 μg g^{-1} water-soluble Cr(VI) for a 20 mg sample. Hence, the LOV microcolumn was proven to be suitable for separation purposes in on-line fractionation/speciation analysis of highly polluted substrates. As a result of the inherent versatility of the μSI-LOV-BI-AAS coupling, environmental solids with variable amounts of available Cr(VI) ranging from the sub-μg kg^{-1} to the mg kg^{-1} level, *i.e.*, above the maximum permissible concentrations for agricultural use, may be automatically treated and further analyzed in the fully enclosed flow set-up.

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MINI-REVIEW

Recent Developments in Automated Determinations of Trace Level Concentrations of Elements and On-Line Fractionation Schemes Exploting the Micro-Sequential Injection—Lab-On-Valve Approach

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Abstract: The determination of trace level concentrations of elements, such as metal species, in complex matrices by atomic absorption or emission spectrometric methods often requires appropriate pretreatments comprising separation of the analyte from interfering constituents and analyte preconcentration. In this context sequential injection (SI) and lab-on-valve (LOV) schemes have proven themselves as superb vehicles to act as front-end microanalytical methodologies, particularly

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when employing solid-phase extraction (SPE) procedures. In this communication, selected SPE-procedures in the bead-renewable fashion are presented as based on the exploitation of micro-sequential injection (μSI-LOV) using hydrophobic as well as hydrophilic bead materials. The examples given comprise the presentation of a universal approach for SPE-assays, front-end speciation of Cr(III) and Cr(VI) in a fully automated and enclosed setup, and the combination of SPE with fractionation schemes of environmentally interesting solid samples (such as soils or sediments) in order to conduct ecotoxicological studies.

Keywords: Sequential injection, lab-on-valve, automation, speciation, metal species, fractionation schemes

INTRODUCTION

Within recent years research efforts in the analytical community have been focused on the determination of trace level concentrations of various elements, particularly metals and metalloids, in complex matrices. To this end, schemes based on the use of flow injection (FI), sequential injection (SI) systems, and lately, specifically, the extension of SI, the so-called micro-sequential injection–lab-on-valve (μSI-LOV) approach, have been exploited as hyphenated with atomic absorption or emission spectrometric detection techniques such as flame atomic absorption spectrometry (FAAS), electro-thermal atomic absorption spectrometry (ETAAS), and inductively coupled plasma-mass spectrometry (ICP-MS) in order to obtain optimal sensitivity and selectivity. Although these instrumental methods are some of the most sensitive detection devices available, they are, to some extent, prone to spectroscopic and/or non-spectroscopic interferences, especially if the sample matrix contains high levels of salts. Therefore, it is often necessary to subject the sample to appropriate pretreatment procedures, that is, to separate the analyte species from potentially interfering matrix constituents, permitting accomplishment of analyte preconcentration at the same time, which, indeed, might be advantageous, or even necessary, if minute concentrations are to be determined.

In this context, the fully computer controlled SI- and LOV-systems proved to offer themselves as unique and highly advantageous front-end vehicles to implement such pretreatment methodologies. All required unit operations can be effected in an enclosed and automated fashion and under strictly controlled conditions (Lenehan et al. 2002; Hansen and Wang, 2002, 2004; Ruzicka 2000; Wang and Hansen 2003). An additional advantage is that aspiration and propelling of sample and reagent solutions can be effected extremely accurately via the use of the incorporated syringe pump, and that merely minute amounts of solutions are required, which in turn means that only small amounts of wastes are generated.

Various on-line pretreatment procedures have been developed, including solvent extraction (Fang, 1993a; Miró et al. 2005), solvent

extraction/back-extraction (Hansen and Wang, 2002; Wang and Hansen 2005), solid-phase microcolumn extraction involving ion-exchange, chelation, or hydrophobic interactions (Trojanowicz 2000; Fang et al. 1996; Fang 1993b; Vereda-Alonso et al. 2001), hydride and vapour generation (Tsalev 1999; Pohl 2004; Wan et al. 2006), precipitation/coprecipitation (Wang and Hansen 2005; Fang, et al. 1996; Vereda-Alonso et al. 2001; Wang et al. 2005), and sorption of neutral complexes in polytetrafluorethylene (PTFE) knotted reactors (Ivanova et al. 1998; Yan and Jiang 2001). In this communication focus will be placed on the use of solid-phase extractions (SPE). Conventionally, SPE-procedures have been implemented by the use of permanent packed column reactors, yet in long term operation these reactors are prone to problems due to the following: (i) progressively tighter packing of the column material (e.g., beads or turnings) resulting in an increase of back pressure; (ii) carry-over effects; (iii) variations in sorbent volume; (iv) malfunctions of the active entities, including loss of functional groups, the latter being a common problem for reagent impregnated bead materials; and (v) surface deactivation due to irreversible interfering species. All these problems can be eliminated by adapting the concept of renewable surfaces, or as it has been termed, bead injection (BI), that is, where the solid-phase material, if called for, can be renewed for each analytical cycle. This is readily feasible in the LOV-approach, which in this context constitutes it to act as an ideal front-end for appropriate sample manipulation prior to the introduction of the analyte species into the detector used.

However, in order to be operated in the μSI-BI-LOV mode there are some stringent requirements to the solid-phase materials employed: They must be (a) perfectly spherical (i.e., in the form of globe-shaped particles); (b) uniform in size distribution (falling within a range of 40–150 μm); and (c) possess a density close to that of water.

Basically, there are two types of beads that are applicable: hydrophobic and hydrophilic materials. An example will be given later of the application of each type of material for determination of metals ions, as exemplified via recent research activities. Therefore, the content of this communication—which should be regarded as a sequel to our earlier published mini-review (Hansen and Wang 2004)—will firstly describe the use of hydrophobic bead surfaces as reagent carriers for presenting a universal approach for selective trace metal determinations as exemplified by the assay of Cr(VI) (Long et al. 2005a). And then the use of a hydrophilic bead material for speciation analysis of ultra trace levels of Cr(III) and Cr(VI) via in-line chemical reduction of Cr(VI) to Cr(III) will be presented (Long et al. 2005b).

Finally, and in line with current research interests on determination of various constituents (e.g., metals) in complex matrices, a survey of our recent investigations into determining metals in soils via fractionation schemes by means of a newly constructed microcolumn incorporated within an SI/LOV-system will be offered (Chomchoei et al. 2004, 2005; Long et al. 2006). This research activity was originally spurned by the desire to

develop a direct LOV procedure for fractionation studies in order to assess the current impact of trace metals in solid substrates (e.g., soils or sediments). However, as discussed later in detail, it was impossible to reproduce the manipulation of the nonhomogenous solid particles within the LOV microconduits as such. As a result, the custom-built extraction microcolumn was utilized as an external module and subsequently proven to be most effective. Integrated into the LOV system, and hyphenated with ETAAS, its practical applicability will be described for in-line dynamic extraction and automated determination of readily bioavailable Cr(VI) in soils as effected under simulated environmental scenarios.

DETERMINATION OF METAL IONS BY μSI-BI-LOV-ETAAS UTILIZING HYDROPHOBIC SURFACES

The determination of metal ions by solid phase extraction via sorption/elution of metal chelates on hydrophobic surfaces inherently requires the generation of non-charged complexes. This approach entails specific advantages as compared to the use of nondiscriminating surfaces such as ion-exchangers, because improved selectivity can be obtained via intelligent choice of the (predominantly organic) complexing agent employed to generate the metal chelate, which, in turn, might imply higher tolerance to potentially interfering cations, because the bead surface will only retain/adsorb noncharged molecules and not ions.

In the previously described on-line procedures the sample containing the target metal ion is usually mixed with the selected complexing reagent and the chelate formed is retained on the hydrophobic beads contained within a packed column reactor (Miró et al. 2003; Long et al. 2004). Following appropriate washing of the loaded beads, the chelate is then stripped out by a suitable eluent and the metal is determined in the attached detection device, generally FAAS, ETAAS, inductively coupled plasma-atomic emission spectrometer (ICP-AES), or ICP-MS. Although suitable in many instances, this approach might give rise to some problems, especially related to the kinetics of the formation of the chelate itself and of its concurrent retention on the bead surface. However, these problems can be solved by resorting to a combination of two schemes (see Fig. 1):

Firstly, by implementing off-line pretreatment of the hydrophobic beads with the selected ligand, advantage can be taken of the fact that the conditions for the impregnation step, such as the pH-value and the time frame for strong sorption of the organic ligand onto the hydrophobic surfaces, readily can be optimized. And then using the reagent-loaded sorbent for complexation/retention of metal species, we again can manipulate the conditions so that they are optimal for on-line operation (e.g., pH). The advantage is that kinetic problems associated with chelate adsorption hereby can be vastly reduced or even eliminated.

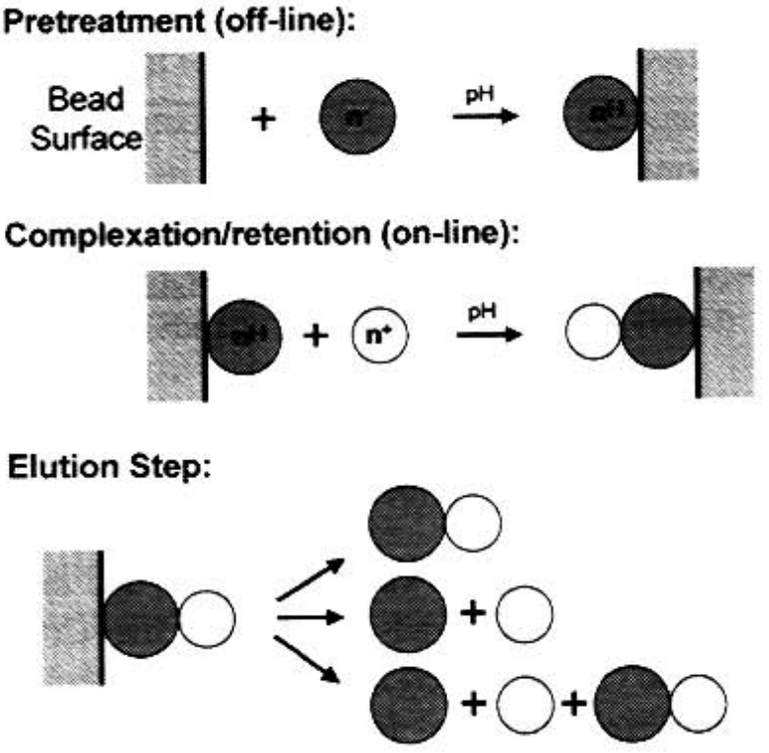

Figure 1. The concept of the universal approach, where the hydrophobic beads initially are preimpregnated off-line with the selected ligand, advantage being taken of operating under optimal reaction conditions to affix the ligand. These pretreated beads are then used for on-line retention of the analyte metal species, the elution and subsequent determination of the metal being unaffected of the mechanisms involved in the liberation of the retained chelate, because the beads are renewed for each sample cycle.

Secondly, in the elution step, where the chelate is eluted from the hydrophobic surfaces, we have added options, because it does not matter how the stripping process actually occurs. Thus, whether it involves the release of the whole complex as such, or a splitting up of the complex (where the ligand might either remain on the bead surface or dissolve into the eluent medium), or as a combination of both, is of no concern, because in the LOV configuration the beads can readily be renewed for each measurement cycle. This is why we have opted to call this pretreatment scheme of the beads the universal approach, because whatever ligand, which can be adsorbed, is applicable—irrespective of the kinetics involved and the elution protocol needed for final quantification.

A very good example to demonstrate this approach is the determination of Cr(VI) using spherical, hydrophobic beads consisting of poly (styrene-divinylbenzene) containing pendant octadecyl moities (C_{18}-PS/DVB) pre-impregnated off-line with 1,5-diphenylcarbazide (DPC). Although the determination of Cr(VI) with DPC is a well-known and widely used procedure in batch assays, it was found virtually impossible to implement the chemistry on-line with the naked hydrophobic beads. This is because the rate-limiting step actually is the adsorption of the ligand onto the bead surface, which is a very slow process. Experimentally, it was thus observed that it takes ca. 30 min in a 5% (v/v) methanol/water medium to be accomplished, as revealed by following the progressively more intense reddish color attained by the beads. Therefore, it is evident that the use of

preimpregnated beads in the LOV microconduits is particularly advantageous for this application.

The reaction between Cr(VI) and DPC is actually rather complex as shown in the following formula:

$$2CrO_4^{2-} + 3H_4L + 8H^+ \longrightarrow \underset{\text{(beads)}}{2[Cr(VI)\text{-}H_4L]} + H_4L$$

$$\downarrow$$

$$[Cr(HL)_2]^+ + Cr^{3+} + H_2L + 8H_2O$$

(beads)

-that is, first a complex is formed between the Cr(VI) and the carbazide (H_4L) affixed on the preimpregnated beads, in which the Cr(VI) oxidizes the carbazide to carbazone (H_2L), which in turn results in half of the generated Cr(III) being complexed by the immobilized carbazone, and, hence, retained on the beads, while the other half is wasted. In Fig. 2 the LOV-manifold used for the actual analytical procedure is shown. The entire set of analytical steps is preprogrammed and computer operated via the LOV software and can be summarized in detail as follows:

Following cleansing of the system with diluted nitric acid, a metered volume of aqueous sample is aspirated from the common port 5 and stored

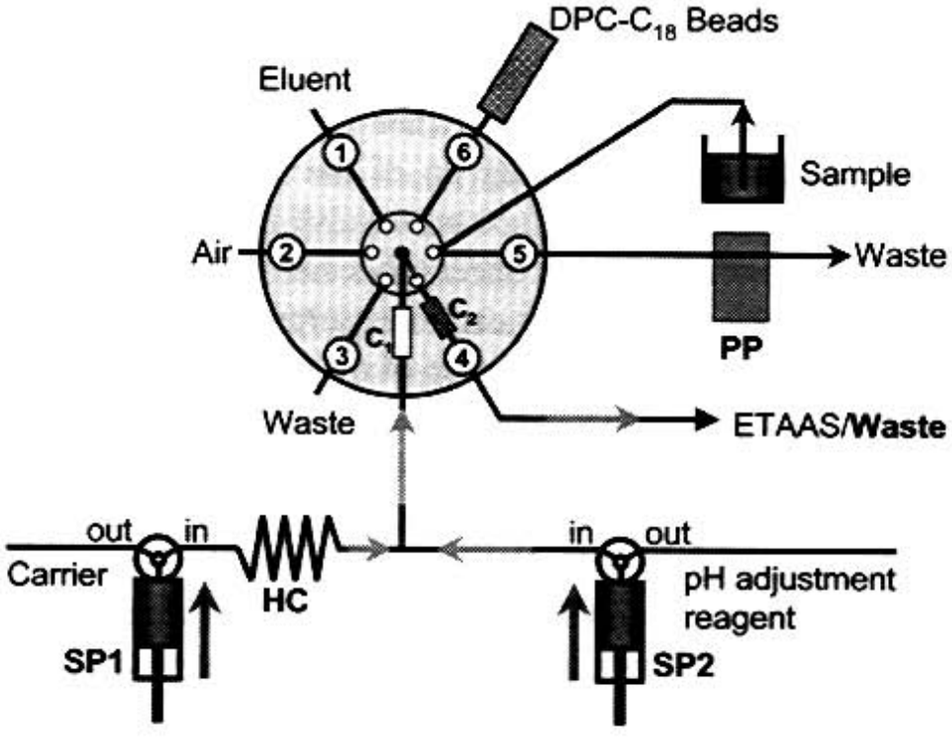

Figure 2. LOV-manifold used for determination of Cr(VI) by solid phase extraction of Cr(VI) via reaction with diphenylcarbazide (DPC) using hydrophobic DPC loaded–C_{18} beads. For explanatory details, see text. The figure shows that step where sample solution (propelled by syringe pump SP1) and pH-adjustment reagent (propelled by syringe pump SP2) are merged and transported to column position C_2, where the target species is retained by the DPC immobilized on the contained beads. HC, holding coil; PP, peristaltic pump.

Table 1. Selected analytical data for the LOV-system using DPC-loaded C_{18}-PS/DVB beads with ensuing determination by ETAAS

Regression equation (Cr, $\mu g\,L^{-1}$)	0.2692[Cr] + 0.0022
Linear range ($\mu g\,L^{-1}$)	0.12–1.5
Sample volume (mL)	2
Retention efficiency (%)	38.8
Enrichment factor	12.9
Detection limit ($\mu g\,L^{-1}$; 3σ)	0.03
Precision (%)	3.8

in the holding coil [HC; the peristaltic pump (PP) connected to port 5 ensures filling of connecting lines between individual samples, and thus prevents carry-over]. Next, a minute, well-defined amount of beads are aspirated (from port 6) into column position C_1 (this column and column C_2 are furnished at the ends with small stoppers that will retain the beads yet allow solutions to flow freely). The beads are then loaded with a merged stream consisting of the sample previously stored in the HC and of the pH-adjustment reagent (5 M HNO_3) in order to facilitate the reaction, which must take place in acid solution, yet the acidity should not be excessive, because this will influence the ability to withhold quantitatively the target metal species onto the beads. Besides, a too high acidity might cause interconversion of Cr(VI) to Cr(III). During sample loading the beads are transported from column position C_1 to C_2 as shown in Fig. 2. Afterwards, a metered volume of methanol (40 µL in order to be accommodated into the platform of the graphite tube of the atomizer) is aspirated from port 1 and into the HC. Subsequently it is propelled forward in an air-segmented fashion to strip both the chelate and the immobilized reagent off the beads and transport the eluate into the detector. Finally, the used beads are discarded, which is effected by first transporting them back to column position C_1 and then to waste via port 3, since it cannot be done directly from C_2 to port 3, because all communication between the LOV and the outside world is effected via the central communication line.

Table 1 shows the analytical performance of the developed procedure for Cr(VI) determination by exploiting hydrophobic beads with physically immobilized DPC. As seen, it features a very low limit of detection and excellent repeatability for the renewable sorbent approach.

DETERMINATION OF METAL IONS BY µSI-BI-LOV-ETAAS UTILIZING HYDROPHILIC SURFACES. SPECIATION OF Cr(III) AND Cr (VI)

The hydrophilic bead material used for the speciation of Cr(III) and Cr(VI) consists of Chelating Sepharose, which is an agarose-based material (mean

bead size of 90 μm) with covalently immobilized iminodiacetate moieties, that is, it can complex and retain Cr(III) ions. The procedural concept is as follows: Cr(III) is determined directly; the sum of Cr(III) and Cr(VI) is quantified via in-line reduction of Cr(VI) to Cr(III), that is, Cr(VI) is determined by difference. The operational procedure can schematically be outlined as depicted in Fig. 3.

First, a well-defined volume of aqueous sample solution is aspirated for Cr(VI) reduction. The reduction is a slow process, so while it occurs, another sample aliquot is aspirated for Cr(III) determination, that is, Cr(III) is preconcentrated on the Sepharose beads and subsequently eluted and quantified. Thereafter, the reduced sample is processed on-line and the total concentration of Cr, that is, the sum of Cr(VI) and Cr(III), is determined. A crucial factor is to identify a reducing agent that can reduce effectively Cr(VI) to Cr(III) in-line. After testing a number of reagents, it was found that hydroxylamine was optimal, but the reaction is rather slow, although

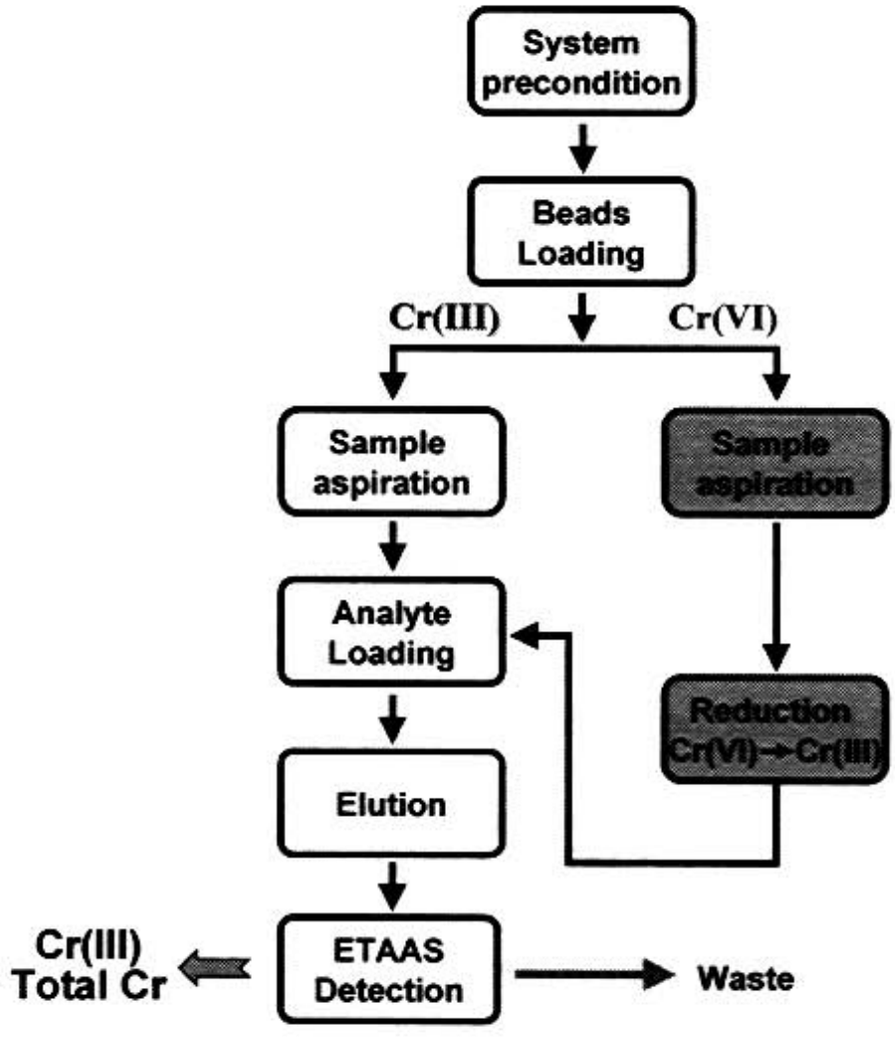

Figure 3. Flow chart of the LOV-procedure to which the Cr(III) and the Cr(VI) species, present in the original sample solution, are subjected. While the Cr(III) ions are separated/preconcentrated on the chelating Sepharose beads and subsequently eluted and quantified by ETAAS, the Cr(VI) ions are reduced to Cr(III) by hydroxylamine (in an open reaction coil, as shown in Fig. 4), and afterwards treated as the native Cr(III) ions. Reproduced from (Long et al. 2005b) by permission of the Royal Society of Chemistry.

over ca. 4 min it leads to a constant and reproducible conversion. However, this is perfectly compatible with the time required by the ETAAS to run through the entire temperature program.

Therefore, the whole procedure, which was implemented in the LOV-manifold depicted in Fig. 4, could be made to proceed as follows: Firstly, a metered amount of chelating beads is aspirated into column position C_1 and then transferred to C_2. An aliquot of the sample for Cr(VI) reduction is subsequently aspirated into the holding coil (HC), whereafter the sample is mixed with the reducing agent (hydroxylamine) and transported to the open reaction coil (RC) and left there for 4 min. In the meantime a metered sample solution for Cr(III) determination is aspirated into the HC, and then loaded onto the beads within column position C_2 (this is the position shown in Fig. 4). A minute, well-defined volume of eluent (0.1 M HNO_3) of the order of 40–50 μL is aspirated from position 2 and stored in the HC and then used for eluting the beads, the eluate being transported to the detector for Cr-quantification (during transport the eluate is sandwiched by air segments in order to preserve its identity). Hereafter, the reduced sample solution is transported back from the RC and into the HC for total Cr determination, and then, as earlier, subjected to the same treatment as the Cr(III)

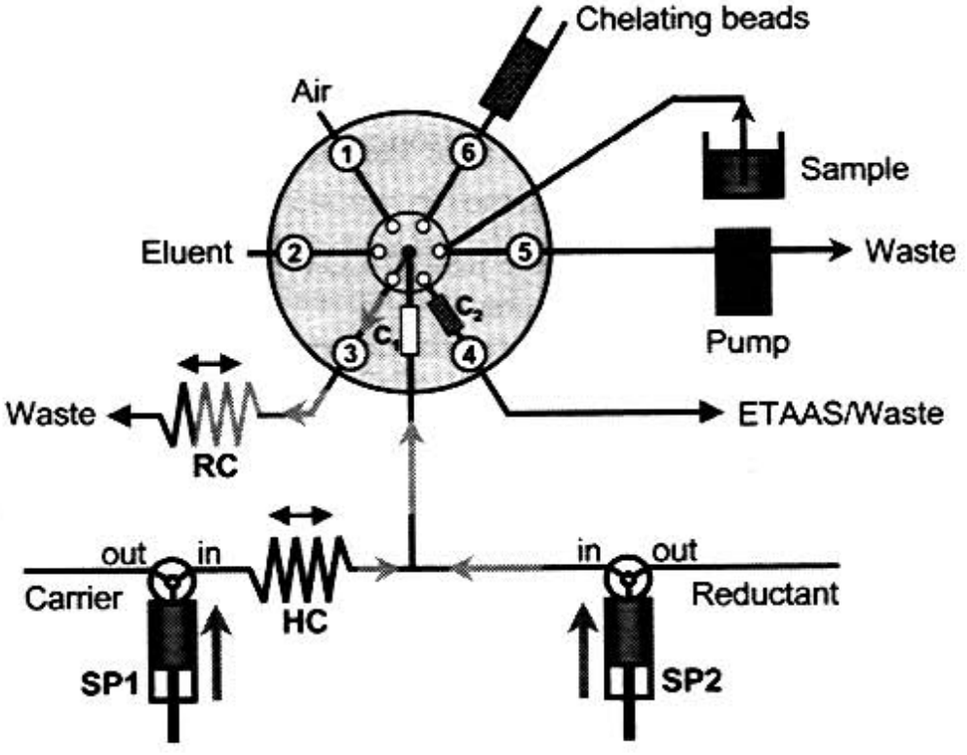

Figure 4. Schematic drawing of the LOV-manifold for Cr(III) and Cr(VI) speciation by solid phase extraction using hydrophilic Sepharose beads. The figure shows that step where sample solution (propelled by syringe pump P1) and reductant (propelled by syringe pump SP2) are merged and transported to the reaction coil, RC, where it is residing for 4 min to reduce Cr(VI) to Cr(III). In the meantime, the Cr(III) in the sample is preconcentrated on the beads placed in column position C_2, and afterwards eluted and quantified, whereupon the reduced sample is subjected to a similar procedure. Adapted from (Long et al. 2005b) by permission of the Royal Society of Chemistry.

aliquot, that is, the beads in column position C_2 are loaded with the reduced sample, eluent is aspirated, the beads are eluted, and the total Cr-concentration is determined.

In Table 2 the analytical performance data for the system is shown. As can be seen, the enrichment factor for Cr(III) is very high (62)—allowing for a very low detection limit—while the enrichment factor for Cr(VI) is somewhat lower (42). This is due to the fact that the reduction efficiency is only 68% ($62 \times 0.68 = 42$), but since it is constant and reproducible it does not impair the measurement due to the exact and reproducible timing that is ensured.

EXPLOITATION OF μSI-BI-LOV AS A MINIATURIZED APPROACH FOR ON-LINE HANDLING AND FRACTIONATION OF SOLID SAMPLES

As discussed in the previous sections, the third generation of flow injection analysis has proven to constitute an excellent avenue for automation and miniaturization of liquid-phase assays and on-line handling of sorbent materials in a fully renewable mode in solid-phase extraction (bead injection) and sample clean-up schemes. Therefore, one might assume that the use of LOV for manipulation of solid samples would be an immediate and straightforward extrapolation of the bead-injection methods. Experimental results revealed, however, that the manipulation of environmental solids, such as soils and

Table 2. Analytical performance of the μSI-BI-LOV-ETAAS system using on-line reduction for Cr(III) and Cr(VI) speciation

Parameter	Cr(III)	Cr(VI)
Regression equation	1.0792 [Cr(III), $\mu g\,L^{-1}$]	0.7380 [Cr(VI), $\mu g\,L^{-1}$]−0.0008
Correlation coefficient	0.9988	0.9990
Linear range ($\mu g\,L^{-1}$)	0.02–0.28	0.035–0.4
Sample volume (mL)	1.8	1.8
Loading flow rate ($mL\,min^{-1}$)	4.5	4.5
Maximum injection throughput (h^{-1})	12	8
Eluent volume (μL)	25	25
Retention efficiency (%)	86	—
Reduction efficiency (%)	—	68
Enrichment factor	62	42
Concentration efficiency (min^{-1})	12.4	5.6
Detection limit ($\mu g\,L^{-1}$) (3σ)	0.010	0.020
Repeatability (%, 0.2 $\mu g\,L^{-1}$, n = 7)	2.4	2.2
Reproducibility (%,0.2 $\mu g\,L^{-1}$, n = 6)	4.7	4.5

sediments in a forward-backward fashion in the integrated microconduits of the LOV unit, is not a simple task. This is a consequence of the large size distribution of the solid particles, shape heterogeneity, and the high solid to suspending solution density ratio that causes a prompt settlement of the sample at the bottom of the external reservoir (i.e., plastic syringe) mounted atop the micromachined module—in contrast to the stable suspension of the easy-to-handle, perfectly spherical beads commonly used in on-line solid-phase LOV extraction. Slurry injection strategies, based upon both mechanical and uninterrupted stirring of the solid suspension in dedicated chambers and continuous recirculation of the sample (even in the presence of surfactants) through the common channel port of the unit (port 5 in Figs. 2 and 4), did not give rise to improved analytical performance owing to the hindrance of repeatable aspiration of minute, well-defined portions of sample into the LOV cavities. In addition, the limited capacity of the cavities (<20 mg soil) restricted the potential applicability of the third generation of flow injection to highly homogeneous solids in order to ensure sample representativeness.

To tackle the previously mentioned shortcomings, the versatility of the μSI-LOV approach in terms of accommodation of peripheral modules at will according to the requirements of the analytical assay was exploited for on-line treatment and analysis of solid samples as contained in a specially designed micro-cartridge (Chomchoei et al. 2004). One interesting analytical application of this arrangement is the development and characterization of flow-through sequential extraction schemes for ascertaining the bioavailability, mobility, and thus toxicity of trace elements in solid substrates by attacking defined chemical forms and metal soil-phase associations (Chomchoei et al. 2004, 2005; Miró et al. 2005). The major asset is the capability of investigating the leachability of targeted species by the action of well-accepted reagents placed at the external ports of the multiposition valve in a continuous, rather than static, extraction mode aimed at mimicking environmental processes more correctly than classical methods that solely offer equilibrium-based information. Additional advantages of automated flow-through dynamic methods over their batchwise counterparts involve shortening of the extraction protocols, minimization of risks of sample contamination and analyses loses, quantification of the size of analyte pools, appraisal of the efficiency of the extractant, investigation of the influence of readsorption/redistribution phenomena, and most importantly, their capability of supplying a detailed insight into the kinetics of the leaching process through the recording of the extractograms, i.e., the representation of extracted trace element amounts vs. time or leachant volume (Miró et al. 2005).

However, miniaturized column-based dynamic extraction protocols so far have not received a broad appeal for two reasons: (i) the minute capacity of in-line microcolumns for accommodating the solid sample and the build-up of backpressure for solid amounts $\geq 50-100$ mg; and (ii) the lack of sensitivity

and selectivity of hyphenated systems for ascertaining the most ecotoxicological relevant forms of trace elements in environmental solids, i.e., the most readily leachable fractions, namely, water-soluble, exchangeable, and mild acid soluble.

One means for alleviation of the Achilles' heel of flow-through fractionation methods capitalizes on the redesign of the sample reservoir and flow network. In fact, the replacement of cylindrical containers by dedicated dual-conical microcolumns not only ensures sample representativeness by admitting substrate amounts up to 300 mg without undue pressure increase, but also features fluidized-bed like conditions that result in appropriate mixing between sample and extractant (Chomchoei et al. 2005). Furthermore, the attachment of the sample container at a peripheral port of the multiposition valve facilitates the application of bidirectional flow with the subsequent mitigation of backpressure or clogging effects due to soil compaction, which are frequently encountered in continuous-flow or uni-directional flow injection fractionation manifolds (Miró et al. 2005).

In the context of dynamic metal/metalloid fractionation, recent trends have focused on the on-line hyphenation of flow injection or sequential injection extraction set-ups with atomic spectrometric detectors (namely, FAAS, atomic fluorescence spectrometer (AFS), and ICP-MS) for automated analysis of the continuously generated extracts (Chomchoei et al. 2005; Jimoh et al. 2004; Beauchemin et al. 2002; Dong and Yan 2005). Yet, direct coupling of microcolumn approaches with FAAS and ICP-MS for assessment of easily accessible metal fractions to biota has two major limitations: (i) it is merely applicable to highly contaminated substrates because the concentration of such metal forms in moderately polluted solids is mostly below the detection limit of the spectrometer; and (ii) the reliability and accuracy of the determinations are strongly dependent on the magnitude of the spectral and non-spectral interfering effects caused by the sample matrix itself and/or the high electrolyte content of the extracting reagent.

To circumvent these drawbacks, appropriate on-line treatment of the sample extracts prior to detection is, therefore, called for; and maximum benefit can be taken from the concept of μSI-LOV with renewable solid-phase extraction in the bead-injection fashion. Thus, the third generation of flow injection can be regarded as a promising tool for on-line soil/sediment fractionation with automated matrix isolation and concomitant analyte preconcentration (Long et al., 2006), as exemplified here via accurate monitoring of the content of easily mobilizable hexavalent chromium in soil environments at the sub-low parts-per-million level. The microflow arrangement, which is shown schematically in Fig. 5, integrates dynamic leaching of Cr(VI) using deionized water or artificial acid rain as single extractants; on-line pH adjustment of the extract to minimize undesired Cr(VI)/Cr(III) interconversions under the slight acidic medium of the aqueous extractants; preconcentration of the chromate leached with

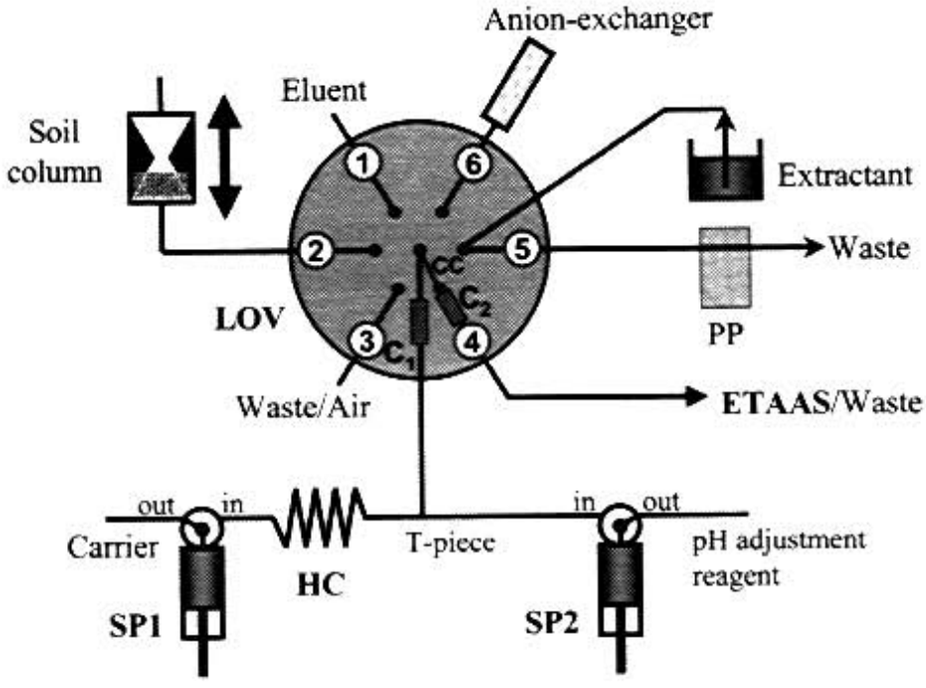

Figure 5. Schematic diagram of the μSI-BI-LOV-ETAAS system for dynamic fractionation of Cr(VI) in environmental solids. The Cr(VI) released from the soil is aspirated into the holding coil (HC) and subsequently mixed with the pH-adjustment reagent (Tris-HNO_3 buffer at pH 8.0) and transported with the previously aspirated beads (Q Sepharose; strong anion-exchanger) into column position C_2, where preconcentration/separation takes place. Afterwards the beads are eluted by 40 μL of 0.5 mol L^{-1} NH_4NO_3/NH_4OH buffer (pH 8.0) and the eluate, via air segmentation, transported to the detector (ETAAS). CC, central communication channel. Reproduced from (Long et al. 2006) by permission of the Royal Society of Chemistry.

concomitant isolation from the matrix constituents and reagent medium by sorption onto strong anion-exchange beads (Q-Sepharose) freshly packed into the microconduits of the LOV assembly; air-segmented elution of the sorbed species that are detected by ETAAS; and finally, withdrawal of the used beads for each step of the multiple extraction protocol to circumvent the progressive sorbent deterioration and the influence of irreversible interferences from the soil matrix. In this configuration, the upright disposition of the microcolumn is intended to withhold the entire substrate in the lower conical cavity of the container for facilitating fluidized bed mixing conditions during the progressive outward pumping of the leaching reagent through the packed column as well as to strip quantitatively the extractant out of the moistened solid whenever the solution is pulled back toward the valve by the reverse motion of the syringe pump.

Figure 6 depicts an example of the multiple-step dynamic extraction profile for assessment of the readily bioavailable content of Cr(VI) in soils as obtained by attacking a moderately polluted soil material (SRM 2709) and various spiked batches with mild extractants. For this particular case, the progressive acidification of the extraction media did not increase the leachability of Cr(VI) from the sample, which is attributed to the efficiency of

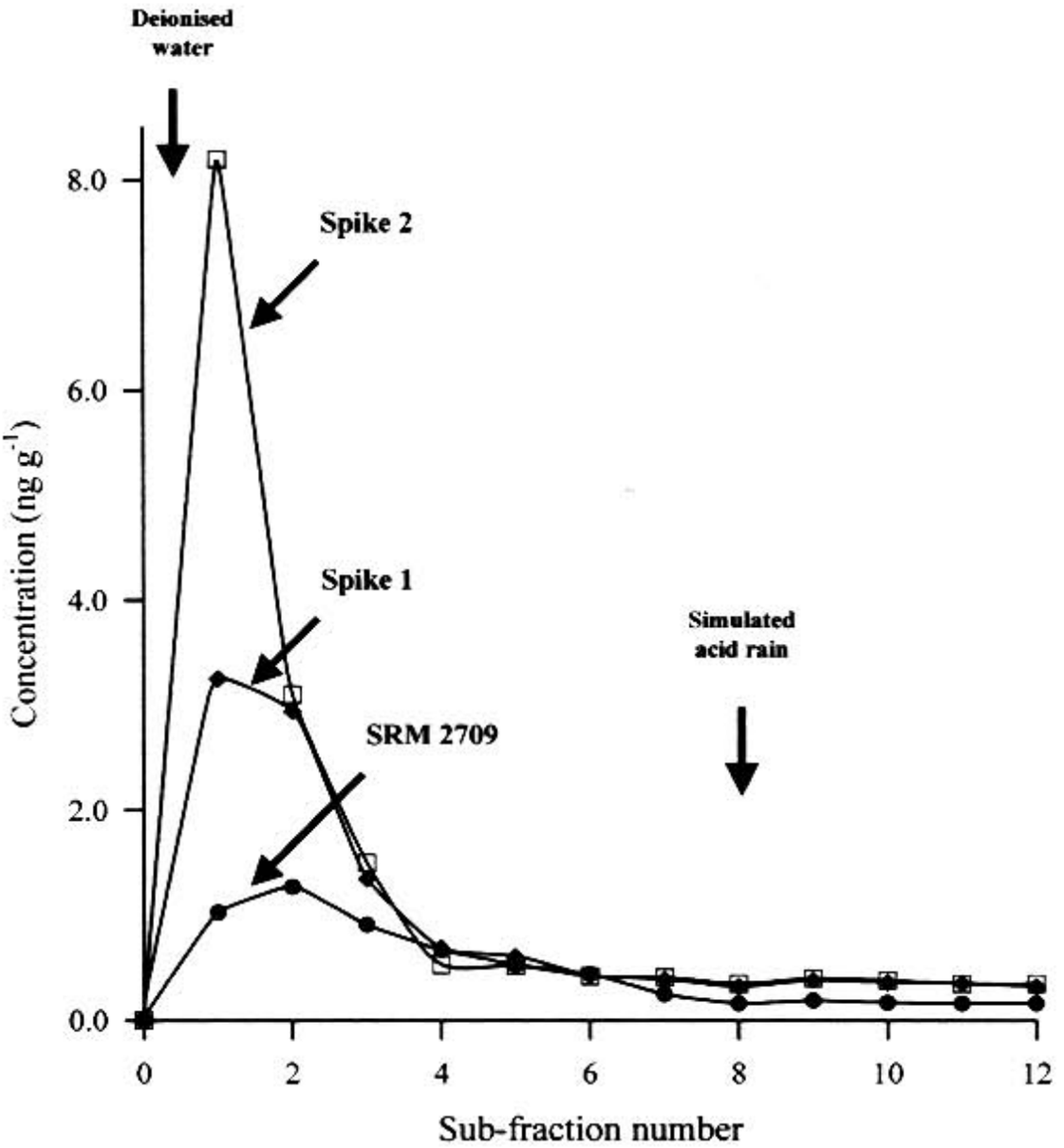

Figure 6. Extraction patterns of readily bioavailable Cr(VI) in San Joaquin soil (SRM 2709, baseline trace element concentrations) and spiked soil samples as obtained from the μSI-BI-LOV microcolumn system using mild extractants, namely deionized water and simulated acid rain. Soil amount, 100 mg; sub-fraction volume, 500 μL; Spike 1, 5.0 ng g^{-1} Cr(VI); Spike 2, 8.0 ng g^{-1} Cr(VI). Reproduced from (Long et al. 2006) by permission of the Royal Society of Chemistry.

distilled water for quantitative removal of soluble (surface bound) chromate in an on-line dynamic mode and the ability of the soil material to raise the pH of the applied extractant, thereby precluding the additional release of sparingly-soluble forms of Cr(VI).

The potential extension of the μSI-LOV analyzer for speciation/fractionation of Cr(VI) and sample clean-up in highly polluted samples have also been assessed by using the miniaturized unit as a front end to FAAS rather than ETAAS. Despite the continuous operation nature of the detection instrument and the discontinuous flow inherent to SI, hyphenation between both setups can be easily realized by interfacing a rotary injection valve for continuous injection of the extracts into the FAAS nebulizer stream (Chomchoei et al. 2005). As a result of the flexibility of the μSI-LOV-AAS coupling,

environmental solids with variable amounts of available Cr(VI) ranging from the sub-$\mu g\,kg^{-1}$ to the $mg\,kg^{-1}$ level, i.e., above the maximum permissible concentrations for agricultural use, may be automatically treated and further analyzed in the fully enclosed flow assembly.

Further research is being currently conducted in our group to miniaturize sequential extraction schemes for trace metals and metalloids in environmental solids exploiting μSI-LOV flow systems.

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ORIGINAL PAPER

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Highly selective micro-sequential injection lab-on-valve (μSI-LOV) method for the determination of ultra-trace concentrations of nickel in saline matrices using detection by electrothermal atomic absorption spectrometry

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Abstract A highly selective procedure is proposed for the determination of ultra-trace level concentrations of nickel in saline aqueous matrices exploiting a micro-sequential injection Lab-On-Valve (μSI-LOV) sample pretreatment protocol comprising bead injection separation/pre-concentration and detection by electrothermal atomic absorption spectrometry (ETAAS). Based on the dimethylglyoxime (DMG) reaction used for nickel analysis, the sample, as contained in a pH 9.0 buffer, is, after on-line merging with the chelating reagent, transported to a reaction coil attached to one of the external ports of the LOV to assure sufficient reaction time for the formation of $Ni(DMG)_2$ chelate. The non-ionic coordination compound is then collected in a renewable micro-column packed with a reversed-phase copolymeric sorbent [namely, poly(divinylbenzene-co-N-vinylpyrrolidone)] containing a balanced ratio of hydrophilic and lipophilic monomers. Following elution by a 50-μL methanol plug in an air-segmented modality, the nickel is finally quantified by ETAAS. Under the optimized conditions and for a sample volume of 1.8 mL, a retention efficiency of 70 % and an enrichment factor of 25 were obtained. The proposed methodology showed a high tolerance to the commonly encountered alkaline earth matrix elements in environmental waters, that is, calcium and magnesium, and was successfully applied for the determination of nickel in an NIST standard reference material (NIST 1640-Trace elements in natural water), household tap water of high hardness and local seawater. Satisfying recoveries were achieved for all spiked environmental water samples with maximum deviations of 6 %. The experimental results for the standard reference material were not statistically different to the certified value at a significance level of 0.05.

Keywords Brines · Nickel · Lipophilic/hydrophilic beads · Micro-sequential injection lab-on-valve · Preconcentration

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Introduction

Electrothermal atomic absorption spectrometry (ETAAS) is one of the most sensitive, well-developed and popular analytical techniques for the analysis of trace level concentrations of metal elements. However, their direct determination is still regarded to constitute a challenge to analytical chemists when it comes to the analysis of environmental water samples, such as seawater and brines,

which generally contain very low contents of the analyte ions (often sub-μg L^{-1} levels) and inherently high levels of alkaline and alkaline earth elements that can give rise to considerable spectroscopic and non-spectroscopic interferences in the detection step. In this context, a pretreatment procedure involving the separation of the targeted ions from the interfering matrix constituents, plus a concomitant preconcentration of the analyte species to fall within the dynamic operational range of the ETAAS instrument, is often a must.

Compared with the conventional batch mode, flow injection (FI) analysis [1–3] constitutes an attractive approach for automated solution manipulation including on-line sample pretreatment [4–7]. The second generation of FI, that is, sequential injection (SI) [8–10], offers merits such as automation, miniaturization, low consumption of sample and chemical reagents, and hence a minimal production of waste, and, being an enclosed system, low risks of contamination. In addition, it is regarded as a very promising tool for sample separation and preconcentration [11]. Further developments of SI involving the incorporation of a micro-conduit manifold mounted atop a selection valve, the so-called Lab-On-Valve (LOV), greatly extends the functions and versatility of the approach to allow a multitude of unit operations [12].

Solid phase extraction (SPE) [13] is the most attractive sample processing technique that can be readily miniaturized in the LOV unit. In fact, the application of the bead injection (BI) mode [14] in μSI-LOV for SPE has made it a very powerful micro-analytical tool in recent years [12, 15, 16].

Classified by their properties, the sorbent materials employed for μSI-BI-LOV fall into two categories: that is, the hydrophilic ones [17–20] and the hydrophobic ones [16, 21–23]. The most popular hydrophilic sorbents are ion-exchangers, including sulfopropyl-Sephadex and Sepharose beads. The main advantages of these materials are their commercial availability, trouble-free automatic handling within micro-conduit systems (e.g. LOV microchannels) and high retention efficiencies, thus implying high enrichment factors. In addition, they are applicable for a wide range of transition and heavy metals. Their disadvantage resides in the lack of selectivity due to the small differences in stability constants for the various metal ions and, even, between groups of elements. Hence, when such sorbent materials are employed for a sample containing large amounts of alkaline earth elements, their ability to retain a given trace metal becomes impaired by the lack of exchange capacity as a consequence of the saturation of the resin with the interfering ions. Therefore, functionalized sorbents with chelating moieties [13] or tailor-made ion-imprinted polymers (IIPs) [24] have been introduced for selective trace metal preconcentration. Even though the metal recognition capabilities of IIPs are well-established, the poor solubility of the analyte (template) in the imprinting mixture and the bleeding of unleached template with time limit their actual applicability [24]. In addition, due to difficulties associated with the synthesis of these materials, their commercial availability is rather scarce.

An appealing alternative to ion-exchange or ion-recognition processes is based on the exploitation of hydrophobic sorbents for the uptake of non-charged chelates generated by the reaction of the analyte with an appropriate, ideally selective, complexing reagent. The main benefit of this kind of sorptive material rests in the potentially high selectivity that can be obtained through an intelligent selection of the chelating reagent; that is, only the target ion, or preciously few other ions, are complexed and thus retained. Any positively or negatively charged matrix ion cannot be collected by the sorbent material. Even if some ions happen to be retained on the surface of the beads by weak interactions, they can be stripped out by the carrier solution itself during a cleansing step following sample loading. Another asset is that the chelate readily can be eluted by water-miscible organic solvents via a change in the polarity of the carrier solution rather than by employing strong acids to release the metal bound to the organic ligand. On-line LOV-based micro-fluidic procedures using this highly selective technique coupled to detection by atomic absorption or emission spectrometry have attracted extensive research interest in recent years [16, 21, 22]. However, a problem is to reconcile the disparity between the hydrophobic properties of the beads and the environment of the aqueous solution used, which sometimes makes the handling of the sorptive entities in the micro-channels of the LOV troublesome. Consequently, appropriate measures (e.g. bead re-circulation under continuous stirring and repetitive bead disposal protocols) must be taken to enhance the analytical performance of the bead-injection (sorbent renewable) approaches [22].

Precipitation might be regarded as an interesting option to SPE for on-line separation and preconcentration in flow systems [25]. Among the various techniques used for collecting the precipitate, knotted reactors (KR) [5, 7, 26, 27] feature low-flow resistance and high concentration factors, but at the expenses of low retention efficiencies (30–60 %). Membrane filters [28–30] have also been employed for precipitate collection, and despite their high efficiency they frequently give rise to the generation of flow impedance due to the accumulation of fine particles. Wang et al. [23] have recently reported that the use of a renewable micro-column in μSI-LOV, as employed to collect a cadmium hydroxide precipitate through surface adsorption, provided a promising approach to eliminating the above-mentioned drawbacks associated with the buildup of flow resistance and malfunctions of the sorbent surface.

It is well known that Ni(II) and dimethylglyoxime (DMG) share a very characteristic, selective reaction and that a neutral coordination compound is produced. It has been shown that octadecyl (C_{18})-chemically modified silica gel in a permanent packed column mode is a suitable medium for the collection of the $Ni(DMG)_2$ precipitate through hydrophobic interactions [31]. However, the use of the permanent column mode is often prone to problems due to the presence of other neutral organic compounds in the sample which can also be retained on the beads and give rise to some irreversible changes of the sorbent surface.

In the investigation reported here, we used a μSI-LOV system in the renewable bead-injection SPE mode to selectively determine Ni(II) by means of a lipophilic/hydrophilic copolymeric sorbent (poly(divinylbenzene-co-N-vinylpyrrolidone)) for the extraction and preconcentration of $Ni(DMG)_2$. The rationale for this choice is that the material, as opposed to conventional hydrophobic sorbents such as polytetrafluoroethylene and octadecyl-chemically modified silicagel [16, 22, 32], not only possesses a superior reversed-phase retention capacity but also entails a trouble-free handling in the μSI-LOV micro-conduits. Although the polymeric material is a commonly used SPE sorbent for the isolation of both neutral and acidic/basic organic pollutants prior to liquid or gas chromatography [33, 34], to the best of our knowledge its analytical application as a sorptive preconcentration medium for the isolation and preconcentration of metal chelates has not been reported to date.

Experimental

Instrumentation

The μSI-LOV-ETAAS system consisted, as depicted in Fig. 1, of the following components: a sequential injection system (FIAlab-3,000, Bellevue, Wash.), a home-made integrated LOV unit, an atomic absorption spectrometer (AAnalyst 600; Perkin Elmer, Foster City, Calif.) furnished with a longitudinal Zeeman background corrector and a transversely heated graphite furnace, equipped with pyrolytically coated graphite tubes, and an AS-800 autosampler. A wavelength of 232.0 nm with a spectral bandpass of 0.2 nm and an operating current of 25 mA were set for the nickel hollow cathode lamp (Perkin Elmer). The temperature program for the graphite atomizer is listed in Table 1. The signals were recorded in the integrated (peak area) mode.

The sequential injection system comprised a six-port selection valve (SV) atop of which was mounted the central sample processing unit named LOV. The LOV was connected to a holding coil (HC), a reaction coil (RC), high-precision bi-directional syringe pumps (SP1: 5 mL; SP2: 2.5 mL) and a peristaltic pump. The two-way valves at the heads of the syringe pumps facilitated the communication of each syringe either with an external container, which provided carrier or auxiliary reagent, or with the manifold. A PEEK T-connector was used for connecting SP2 to RC. The volumes of HC and RC were 3.0 mL and 2.5 mL, respectively, corresponding to 170 cm and 142 cm of 1.50-mm (i.d.)/2.10-mm (o.d.) polytetrafluoroethylene (PTFE) tubing, respectively. All other connecting tubes were made from 0.50-mm (i.d.)/1.66-mm (o.d.) PTFE tubing. The peristaltic pump, furnished with Tygon tubing of 1.22 mm (i.d.)/2.80 mm (o.d.), was utilized for filling the conduit from the external sample reservoir to port 5 with fresh sample solution prior to the analysis of the ensuing sample.

The dedicated LOV processing unit (diameter: 5 cm, thickness: 1 cm), which was made from hard polyvinyl chloride (PVC), contained a central port and six micro-channels [1.66 mm (i.d.)/12.0 mm (length)]. The communication between them was effected through a central micro-channel (CC) in the SV. PEEK stoppers (Upchurch Scientific, Oak Harbor, Wash.) were introduced in port 4 as well as in the central port connecting HC with CC in order to facilitate the formation of micro-columns C_1 and C_2. The stoppers, having a dimension slightly smaller than that of the channel, allowed the solution to flow freely while entrapping the beads in the respective cavities.

All of the operations of the μSI-LOV system were controlled by the associated FIAlab software (FIAlab

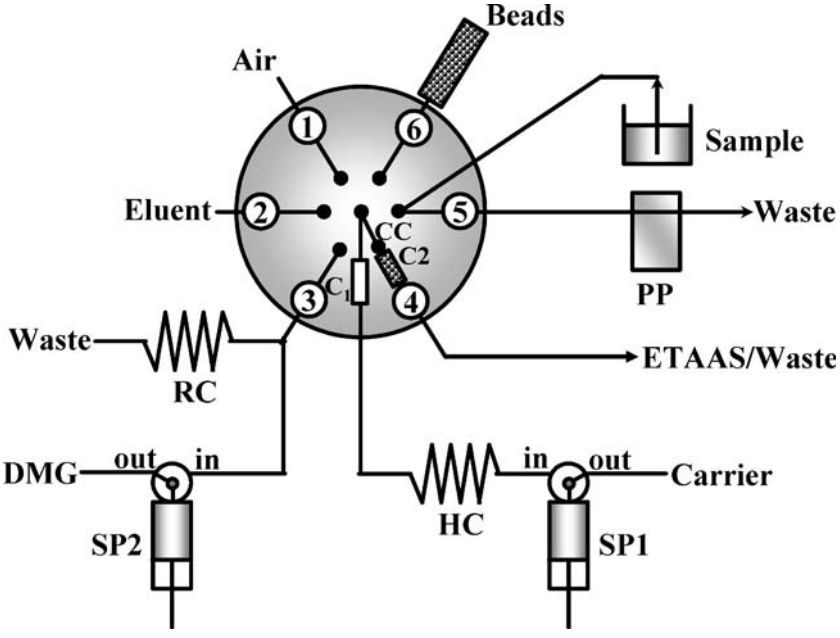

Fig. 1 Schematic diagram of the μSI-LOV-ETAAS system for on-line determination of Ni(II) via precipitation with DMG and preconcentration on poly (divinylbenzene-co-N-vinylpyrrolidone) beads. *Carrier* 0.2 mol L^{-1} ammonium citrate buffer (pH 9.0), *DMG* 1.2 % (w/v) dimethylglyoxime in ethanol, *eluent* methanol, *SP1* and *SP2* syringe pumps 1 and 2, C_1 and C_2 LOV micro-column positions, *HC* holding coil, *RC* reaction coil, *CC* central communication channel, *PP* peristaltic pump, *ETAAS* electrothermal atomic absorption spectrometer

Table 1 Temperature program for the ETAAS determination of nickel

Step	Temperature (°C)	Ramp time (s)	Holding time (s)	Argon flow rate (mL min^{-1})
Drying 1	100	5	20	250
Drying 2	130	5	20	250
Pyrolysis	1100	10	30	250
Atomization	2150	0	5	0
Cleansing	2500	1	4	250

Instruments, Bellevue, Wash.) and synchronized with the commands for the activation of the temperature program of the ETAAS instrument through an intelligent electronic interface [35]. The μSI-LOV system was actually programmed to execute the analytical protocol for the next analysis cycle while the former sample was pyrolyzed and atomized in the graphite furnace.

A digital pH meter (PHM92 LAB pH Meter; Radiometer A/S, Copenhagen, Denmark) was employed for measuring the pH of the solutions.

Reagents and solutions

All reagents were of analytical-grade, and doubly deionized water (18.2 MΩ cm), obtained from a Milli-Q Synthesis A10 system (Millipore, Molsheim, France), was used throughout.

An ammonium citrate stock solution with a concentration of 1.0 mol L^{-1} was prepared by dissolving 22.619 g of salt in 80 mL of water and adjusting the pH to 9.0 by the addition of concentrated ammonium hydroxide prior to the addition of water to a final volume of 100 mL. The carrier was obtained by diluting the 1.0 mol L^{-1} ammonium citrate buffer to 0.2 mol L^{-1} with water. Ni(II) working standard solutions were prepared from a 1000 mg L^{-1} Ni(II) stock solution (Merck, Darmstadt, Germany) by stepwise dilution with the 0.2 mol L^{-1} ammonium citrate buffer. A 1.2 % (w/v) DMG solution was prepared by dissolving 1.2 g DMG in 100 mL ethanol. Methanol was used as eluent.

Poly(divinylbenzene-co-N-vinylpyrrolidone) (Oasis HLB) beads with an average dry particle size of 30 μm and a specific surface area of 800 m^2 g^{-1} were purchased from Waters (Mildford, Mass.). Working suspensions were prepared by moistening 0.1 g of beads with 2 mL methanol. The analytical performance of these copolymeric beads was compared with that of an octadecyl chemically modified poly(styrene-divinylbenzene) material (C_{18}-PS/DVB) (Polysorb MP-1; Transgenomic, Omaha, Neb.) having a mean particle size of 40 μm. Practical slurries (1:20; w/v) were obtained by suspending the beads in 15 % (v/v) ethanol/water. The individual sorbent suspensions were aspirated into a plastic syringe (1.0 mL) which is mounted atop port 6 of the LOV unit. The tip of the syringe is furnished with a piece of PEEK tubing [0.76 mm (i.d.)/1.6 mm (o.d.)] and a conventional PTFE fitting with ferrule to allow for a tight connection to the LOV port.

The environmental water samples, including hard tap water and seawater, were filtered through a 0.45-μm pore size membrane filter, and then 10 mL of the 1.0 mol L^{-1} ammonium citrate pH 9.0 buffer was added to the 40-mL sample.

The standard reference material SRM 1640 (Natural water) was from the National Institute of Standards and Technology (NIST). As a consequence of the high content of nickel (namely, 27.4±0.8 μg L^{-1} Ni), the SRM was prediluted with the citrate buffer so that the final solution contained a concentration of 0.2 mol L^{-1} ammonium citrate buffer (pH 9.0).

Operating procedure

Before starting the analytical sequence, the tubing connecting the eluent reservoir (port 2) to the LOV, the line connecting SP2 to the T-connector and HC were filled with eluent (methanol), complexing reagent (1.2 % DMG) and carrier (0.2 mol L^{-1} ammonium citrate buffer, pH 9.0), respectively.

Table 2 summarizes the operations of the syringe pumps, the LOV positions, the volumes aspirated/dispensed and concomitant flow rates. Each analytical cycle includes seven steps, as described in the following:

Step 1:
System preconditioning. Firstly, both the line of the ETAAS auto-sampler arm and the RC are rinsed with 2000 μL of carrier from SP1. SP1 and SP2 are then loaded with 1750 μL of carrier and 100 μL of complexing reagent, respectively. Thereafter, the central channel is directed to port 1 for aspiration of 400 μL of air. Next, SP1 and SP2 are operated synchronously: SP2 is programmed to discard the first 40 μL of complexing reagent to waste (port 3) in order to minimize the DMG concentration gradient occurring at the tee-confluence, and SP1 is made to deliver 300 μL of air to RC via port 3 for the separation of the remaining solution in RC from the oncoming sample. The last 100 μL of air are left in the HC.

Step 2:
Sorbent preparation. A metered portion of beads is introduced from port 6 to C_1 and then the central channel is connected to port 2 for the aspiration of 400 μL of methanol. The remaining 100 μL of air in HC serve as a spacer between the carrier and methanol solutions to prevent the undesired overlapping of segments. The central channel is then directed to port 4, and a total volume of 1200 μL of solution, including methanol and carrier, is used for rinsing the sorbent material, which now becomes entrapped in micro-column C_2.

Step 3:
Sample pretreatment. SP1 is set to aspirate consecutively an air plug and 1800 μL of sample solution into HC. Before the mixing of sample and complexing reagent, 100 μL of sample is moved to the inlet of RC

in advance through port 3 to ensure that the sample and complexing reagent precisely merge at the mixing point of the T-connector. Afterwards, SP1 and SP2 move synchronously, thereby dispensing simultaneously 1800 μL of sample and 60 μL of complexing reagent into RC.

Step 4:

Analyte separation and preconcentration. With the central channel still directed to port 3, the interdispersed zones are pulled inwards into HC. The central channel then moves to port 4 to effect the collection of $Ni(DMG)_2$ onto the micro-column C_2. Afterwards, 500 μL of carrier are employed to rinse the analyte-loaded beads. A volume of 580 μL of air is next aspirated from port 1 into HC, from which 380 μL are used to direct the remaining solution in the ETAAS autosampler arm line to waste.

Step 5:

Activation of ETAAS. The ETAAS program is activated by a dedicated command in the SI program which allows the tip of the autosampler arm to move into the dose hole of the graphite tube for further delivery of eluate.

Step 6:

Elution. A minute, well-defined volume of methanol (50 μL) is aspirated from port 2. The central channel is then directed to port 4 and SP1 drives the eluent plug at a slow flow rate (10 μL s^{-1}) to pass through the

Table 2 Protocol for the determination of trace level concentrations of nickel by μSI-LOV-ETAAS

Step	Description	SP1 valve[a]	SP2 valve[a]	SP1	SP2	LOV position	Flow rate / μL s^{-1}	Volume /μL
1	System preconditioning							
	(a) Filling SP1 with carrier	Out		Aspirate			200	2000
	(b) Rinsing ETAAS line	In		Dispense		4	100	2000
	(c) Filling SP1 with carrier	Out		Aspirate			200	2000
	(d) Rinsing RC	In		Dispense		3	100	2000
	(e) Filling of SP1 and SP2 with carrier and complexing reagent, respectively	Out	Out	Aspirate	Aspirate		100 (SP1), 50 (SP2)	1750 (SP1), 100 (SP2)
	(f) Aspiration of air into HC	In		Aspirate		1	50	400
	(g) Introduction of air segment in RC and cleaning of the chelating reagent line	In	In	Dispense	Dispense	3	50 (SP1), 50 (SP2)	300 (SP1), 40 (SP2)
2	Sorbent preparation							
	(a) Beads loading	In		Aspirate		6	5	20
	(b) Methanol loading	In		Aspirate		2	50	400
	(c) Rinsing of beads and ETAAS line	In		Dispense		4	50	1200
3	Sample pretreatment							
	(a) Air segment introduction	In		Aspirate		1	50	100
	(b) Sample loading	In		Aspirate		5	100	1800
	(c) Filling the line between LOV and T-confluence with sample	In		Dispense		3	100	100
	(d) Merging sample with complexing reagent into RC	In	In	Dispense	Dispense	3	60 (SP1), 2 (SP2)	1800 (SP1), 60 (SP2)
4	Analyte separation and preconcentration							
	(a) Transportation of sample and reagent back to HC	In		Aspirate		3	50	1960
	(b) Delivery of interdispersed plugs to the LOV micro-column and rinsing of analyte-loaded beads	In		Dispense		4	20 (C_{18})/50 (copolymeric)	2460
	(c) Introduction of air into HC	In		Aspirate		1	50	580
	(d) Filling ETAAS line with air	In		Dispense		4	20	380
5	Activation of ETAAS							
6	Elution							
	(a) Aspiration of eluent	In		Aspirate		2	20	50
	(b) Dispensing of the eluent plug into column C_2	In		Dispense		4	10	50
	(c) Stopped flow for 5 s	In		–		4	–	–
	(d) Delivery of air-segmented eluate into the atomizer	In		Dispense		4	10	400
7	Bead discarding							
	(a) Dispensing carrier to C_2	In		Dispense		4	50	300
	(b) Bead transportation from C_2 to C_1 positions	In		Aspirate		4	50	300
	(c) Delivering of beads to waste	In		Dispense		3	50	300

[a]The position "out" means the connection of SP with the external reservoir, while "in" means the connection of SP with the manifold

analyte-loaded beads. SP1 is then halted for 5 s, whereupon the air-segmented eluate is transported into the graphite tube. Finally, the autosampler arm moves out of the furnace, and the ETAAS instrument continues to run the temperature program.

Step 7:

Bead discarding. The remaining 300 μL of carrier in the HC is dispensed to port 4 for subsequent backward aspiration of the used beads into HC, finally discarding them to the waste through port 3.

Results and discussion

Configuration of the μSI-LOV-ETAAS system

In this work, a hybrid FI-SI protocol is proposed for efficient radial mixing between the sample and complexing agent, thereby overcoming the limited reagent/sample overlapping inherent to conventional SI set-ups for large sample volumes [36]. Preliminary experiments were conducted using unidirectional forward-flow for simultaneous delivery of the sample (60 μL s^{-1}) and complexing reagent (3 μL s^{-1}) towards the beads contained in C_2. Thus, the T-confluence was located just before the micro-column position C_1. However, the analytical signals for 1 μg L^{-1} Ni were not significantly different from blank values. This was attributed to the short reaction time for on-line formation of the sparingly water-soluble chelate, which amounted merely to 2 s for the assembled flow configuration.

An alternative approach to facilitate the development of the reaction until completion is to increase the residence time for the interdispersed segments into the flow network. This can be elegantly performed in SI-manifolds via an auxiliary reaction coil that can be mounted to one of the peripheral ports of the selection valve. Hence, the mixture of sample and complexing reagent might remain there at will by the appropriate flow programming to acquire sufficient reaction time prior to the application of backward-flow for the collection of the generated precipitate onto the packed micro-column. Using the flow rates quoted in Table 2, the total residence time for the mixture, without resorting to stopped flow approaches, amounted to approximately 70 s. As a result, an 8.5-fold higher signal improvement was attained. To assess the dependence of the system's performance on the reaction time, a stopped-flow command was implemented in the SI program. No significant improvement in method's sensitivity was observed for residence times ranging from 70 to 250 s. Therefore, a reaction time of 70 s sufficed for the on-line generation of $Ni(DMG)_2$.

Different physical configurations and materials for RC were investigated. A knotted reactor (KR) provided the best mixing conditions, but, in the present case, gave rise to an impaired performance due to both the build up of back pressure during the flow-reversal aspiration stage and possible retention of the sparingly water-soluble chelate material on the tubing walls; consequently this option was discarded. Experimental results provided evidence for the nonexistence of significant differences in the analytical readouts at a confidence level of 95 % by exploiting either hydrophobic or hydrophilic tubing (namely, PTFE, Nylon and Tygon) as the open tubular reactor. The reason possibly lies in the fact that a coiled tubing with a large inner diameter (i.e. 1.5 mm i.d.) was employed, and the residence time of the mixture in the RC was relatively short, whereby the interactions of the $Ni(DMG)_2$ precipitate with the reactor were negligible.

The use of air-segmentation during the operational sequences was aimed at defining discrete solution segments in the flow network and at concomitantly preventing undesirable dispersion. Thus, the sample segment could be readily transported in a forward-backward fashion without dispersion into the carrier solution. During the preparation and washing of the beads, the air plug was employed to separate the methanol from the carrier in order to prevent the overlapping of solutions, which might cause the unwanted pre-elution of retained analyte during the clean-up step performed with a metered volume of carrier. An air-based sandwich strategy was also exploited in the elution step to meter a minute zone of eluate to satisfy the volume requirements of the graphite tube.

Parameter optimization

Chemical variables

When non-selective sorbents such as ion-exchangers or chelators are used for SPE for highly saline samples, several avenues have been proposed to improve the tolerance level to interfering matrix constituents. One approach relies on the implementation of a special cleansing step after sample loading that involves the removal of the loosely bound interfering ions by an electrolyte with a weak eluting strength, such as ammonium acetate, prior to the elution of the targeted species [37–39]. The main shortcoming is that it requires an additional operational step, and the analyte might subsequently be partially desorbed during the washing step. Masking reagents or pre-separation steps can be also applied for selectivity improvement. All of these measures are tedious and time-consuming, and sometimes the effect is not satisfactory due to the high level concentrations of potentially interfering elements (e.g. alkaline earth metals) in the sample matrix.

The benefit of using hydrophobic sorbents in SPE lies in the possible intelligent selection of the ligand for selective collection of the target species on the sorbent as a non-ionic complex, while all major matrix constituents remain in the liquid phase. Therefore, the adoption of a hydrophobic sorbent is a very promising approach for the analysis of highly saline samples.

Poly(divinylbenzene-co-N-vinylpyrrolidone) is a hydrophilic-lipophilic, water-wettable reversed-phase sorbent that was selected in this work for the purpose of scavenging

non-charged metal chelates via hydrophobic interactions. The commercially available sorbent has an average dry particle size of 30 μm, and the water-wettable beads can be readily captured in the LOV micro-column cavities. Under the microscope they are revealed to have perfectly spherical shapes and a uniform size distribution. In addition, this sorbent provides enhanced retention capacity for non-ionic and moderately polar species as compared with the traditional silica-based SPE sorbents like C_{18} [32, 40, 41].

With the exception of a few metal elements not commonly encountered in environmental waters, such as palladium, dimethyglyoxime (DMG) reacts selectively with nickel ions and forms a non-ionic colloidal precipitate. The mechanism of uptake of this coordination compound by the co-polymeric beads relies mainly on chemical interactions with the bead surfaces in lieu of physical filtration because hydrophilic sorbents, such as ion-exchange resins, render no appreciable collection of the target species. The complexation reaction involves the generation of oxonium ions, whereby the formation of the nickel chelate is favored under a buffered alkaline medium. Hence, the effect of pH on the yield of $Ni(DMG)_2$ formation was investigated, and the experimental results are shown in Fig. 2. As seen from this figure, the analytical performance improved at a pH>8, while pH 9.0 appears to be optimal; consequently, this latter value was chosen. An ammonium buffer was used for pH adjustment, thereby ensuring a high buffering capacity. However, a surplus of ammonia is inappropriate because the competitive complexation reaction of Ni(II) with ammonia might increase the solubility of $Ni(DMG)_2$. As a compromise between the above-mentioned factors, a buffer concentration of 0.2 mol L^{-1} was selected for the remainder of the studies.

To prevent the formation of insoluble oxyhydroxides of metal ions, such as Cr(III) and Fe(III), in real-life samples, which would in turn co-precipitate with the nickel chelate in the alkaline reaction medium and remain deposited on the LOV column, citrate was introduced to selectively form tightly bound soluble complexes with these metals. Ammonium citrate can thus not only serve as a buffer but also as a masking reagent for potential interfering species. Therefore, a 0.2 mol L^{-1} ammonium citrate buffer (pH 9.0) was employed for preparing the entire set of samples and working standards. The complexing reagent solution was prepared by dissolving DMG in absolute ethanol (1.2 %, w/v). To determine the optimal amount of chelating reagent for the on-line nickel precipitation reaction, various sample (1 μg L^{-1} Ni) to reagent (1.2 % DMG) ratios, ranging from 500 to 10, were assayed. It was found that the increase of DMG yielded improved absorbance signals until a ratio close to 20 was reached. Beyond this ratio, the method's sensitivity remained unchanged. DMG is only sparely soluble in water (0.063 g in 100 ml water at 25 °C), so the surplus of reagent might occasion its on-line crystallization into the flow conduits with the subsequent collection of particles onto the LOV packed micro-column, thus decreasing the retention efficiency for the target analyte. In this context, it should also be borne in mind that the Ni $(DMG)_2$ complex is highly soluble in ethanol. Thus, when the ratio of aqueous solution to reagent is affixed to 20, the ethanol content in the flow system is merely 5 % (v/v), while at a ratio of 10 it will be increased to 10 %. To prevent the partial dissolution of the adsorbed $Ni(DMG)_2$ by the sample medium itself, a minute volume of complexing reagent is therefore preferable. For this reason, a mixing ratio of 20 for sample to complexing reagent was employed for further investigations.

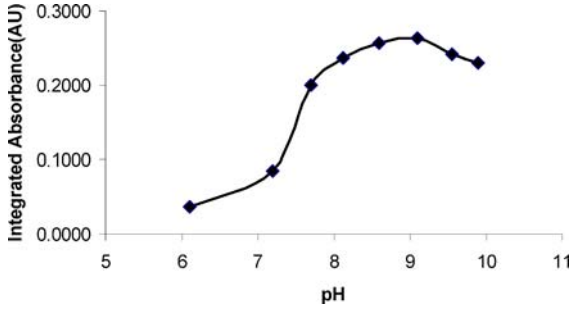

Fig. 2 Influence of sample acidity on the analytical signal recorded. Standard, 1 μg L^{-1}Ni(II); loading volume, 1.8 mL; sample to chelating reagent ratio, 20; loading flow rate, 50 μL s^{-1}, eluent volume, 50 μL

μSI-LOV variables and eluting procedure

To obtain the best performance in the separation and preconcentration of Ni(II) in the LOV unit, the ideal manifold should effectively retain the target species (i.e. the chelate) on the micro-column while permitting the stripping of the analyte quantitatively before its transportation to the detector. To this end, the SI parameters involving loading and elution of the micro-column were investigated.

The flow rates for the uptake of the $Ni(DMG)_2$ precipitate were varied from 10 to 100 μL s^{-1}. The highest integrated absorbances were obtained at flow rates <50 μL s^{-1}, while they decreased by 8 % at a flow rate of 70 μL s^{-1} and by 12 % at 100 μL s^{-1}. Thus, the sample loading flow rate was affixed at 50 μL s^{-1} for further experiments.

A very efficient approach to strip the target metal ion from the collection medium is by breaking the hydrophobic interaction between the solid-phase and the chelate, which can be effected by employing an organic reagent, such as methanol or ethanol. Even if some hydrolyzed metals should remain accumulated on the sorbent after the elution procedure, the analytical performance, as opposed to SPE in permanent columns [31], is not deteriorated with respect to repeatability because the bead injection approach entails the discarding of the used beads and the automatic column re-packing with a fresh portion of sorptive entities for each cycle.

It should be noted that the higher the eluent flow rate, the shorter the contact time between the stripping agent and the sorbent. Furthermore, flow rates >10 μL s^{-1} gave rise to multi-segmented zones, which might cause the incomplete delivery of the air-segmented eluate to the graphite tube. Therefore, a flow rate of 10 μL s^{-1} was finally adopted. A

stopped-flow mode, that is, the eluent is halted for a preselected time interval and remains within the analyte-loaded sorbent column to assure quantitative elution, was also investigated. The experimental results evidenced an improvement in the absorbance signals up to 10 % when the forward-flow elution was replaced by a stopped-flow procedure involving a halting time of 5 s.

An eluent volume of more than 50 μL is rarely allowed to be introduced into the ETAAS atomizer due to the physical dimensions of the graphite tube. In the present application, however, a 50-μL methanol plug was imperative for effective stripping of the sorbed compound since the analytical sensitivity dropped by 20 % whenever the elution was performed with 40 μL of methanol.

To assess whether or not carryover effects were negligible, a further elution step was conducted using an identical eluting protocol prior to sorbent renewal. Sample cross-contamination was calculated to be lower than 2.5 % under the experimental conditions selected, thereby proving that a single 50-μL eluent segment sufficed for effective stripping of the nickel chelate from the sorbent material.

Interferences

The tolerance of the μSI-BI-LOV method to foreign species was evaluated by using a fixed concentration of Ni(II) (0.8 μg L^{-1}) and various amounts of potentially interfering ions. The effect of the most commonly encountered ions in environmental waters – Ca(II), Mg(II), Pb(II), Cu(II), Cd(II), Fe(III), Mn(II) and Co(II) – was thoroughly investigated. The experimental results showed that the interference level was <10 % when the ratio of interfering species, such as Ca(II) and Mg(II), to analyte was below 1×10^6, while a ratio of interferent to analyte below 1×10^4 for Pb(II), Cu(II), Cd(II), Fe(III), Mn(II) and Co(II) caused no significant deviations in Ni(II) recoveries. The high tolerance to alkaline earth elements implies the possibility of direct analysis of hard waters and seawater, with no need for any sample pre-treatment (see below). The tolerance level to interfering ions was compared with another μSI-LOV method in which the ion-exchanger SP Sephadex C-25 was employed for the separation and preconcentration of Ni(II) in environmental and biological samples [42]. In addition to allowing a fivefold higher content of alkaline earth elements, the present method showed a tenfold higher tolerance to Co(II), Mn(II) and Cd(II).

Analytical performance of the μSI-LOV-ETAAS protocol with co-polymeric sorbent beads for the assay of nickel and its comparison with the use of C_{18}-PS/DVB

Under the optimized chemical and μSI-LOV operational parameters, the merit points of the bead-injection protocol exploiting poly(divinylbenzene-co-N-vinylpyrrolidone) as sorptive medium for the determination of nickel are summarized in Table 3, including the regression equation, linear dynamic range, required amounts of sorbent, sample and eluent, sampling throughput, enrichment factor and statistical parameters. The retention efficiency is defined as the ratio of the retained amount of nickel to the total amount of analyte available in the sample. The enrichment factor is determined as the ratio between the linear range sensitivity of the proposed method and that obtained by direct injection of 50 μL of a series of standards. The detection limit is defined as the concentration of analyte corresponding to a signal equal to threefold that of the standard deviation of the blank. Repeatability and reproducibility were obtained by seven consecutive measurements of a 0.8 μg L^{-1} Ni(II) standard using the permanent and renewable column fashion, respectively.

The performance of the poly(divinylbenzene-co-N-vinylpyrrolidone) copolymeric sorbent was compared with that of another reversed phase sorbent, namely, C_{18}-

Table 3 Analytical performance of the μSI-BI-LOV system exploiting N-vinylpyrrolidone-divinylbenzene beads for the determination of Ni(II) and its comparison with C_{18}-PS/DVB as a sorptive material

Parameter	Sorbent	
	C_{18}-PS/DVB	Poly (divinylbenzene-co-N-vinylpyrrolidone)
Regression equation	0.1969 (Ni, μg L^{-1}) +0.0698	0.2057 (Ni, μg L^{-1}) +0.0592
Correlation coefficient	0.9995	0.9978
Linear range/μg L^{-1}	0.2–2	0.2–2
Sorbent volume/μL	20	20
Sample volume/mL	1.8	1.8
Eluent volume/μL	50	50
Loading flow rate/μL s^{-1}	20	50
Sample frequency/h^{-1}	8	10
Retention efficiency (%)	66	69
Enrichment factor	24	25
Detection limit/μg L^{-1} (3σ)	0.04	0.05
Repeatability (%)	4.5	4.8
Reproducibility (%)	7.2	5.6

Table 4 Determination of trace level concentrations of Ni(II) in environmental waters by hyphenation of μSI-BI-LOV with ETAAS

Sample	Added (μg L^{-1})	Found (μg L^{-1})[a]	Recovery (%)
Hard tap water[b] (Kgs.Lyngby, Denmark)	–	0.21±0.01	–
	0.50	0.70±0.04	98
	0.75	0.93±0.06	95
Seawater[a] (Klampenborg, Denmark)	–	0.56±0.01	–
	0.5	1.06±0.06	99
	0.1	1.50±0.04	94

[a]The results are given as the mean of three replicates ± standard deviation
[b]Dilution factor, 40:50

PS/DVB; both showed a very similar performance in most of the parameters listed except for the sample loading flow rate and statistical parameters. Thus, the C_{18}-PS/DVB material needs a relatively lower sample loading flow rate (20 μL s^{-1}) than the co-polymeric material (50 μL s^{-1}) to assure a complete collection of the nickel precipitate. In addition, C_{18}-PS/DVB has a poorer reproducibility. All of these factors can possibly be attributed to the difference in the hydrophobic/hydrophilic nature of the two sorbents. Since all of the micro-fluidic operations are conducted under an aqueous environment, the sorbent with more hydrophilic properties should benefit from this in performance. The hydrophilic monomers of the co-polymeric material can greatly facilitate the interaction between the surface of the beads and the aqueous solution, thereby allowing a faster mass transport on the liquid-solid interface. The reason for the better reproducibility of the water-wettable sorbent should be attributed to the straightforwardness of its handling in the micro-LOV channel system as compared with its counterpart.

Validation

Environmental water samples of relative matrix complexity, including hard tap water and seawater, as well as a certified reference material (SRM 1640) were employed to validate the reliability and accuracy of the proposed method using poly(divinylbenzene-co-N-vinylpyrrolidone) as a renewable sorbent for the separation and preconcentration of ultra-trace levels of nickel. The recoveries obtained for the environmental waters spiked at two different levels of Ni(II) are listed in Table 4. As can be seen from the table, maximum deviations of 6 % were found, thus indicating the absence of multiplicative (non-spectroscopic) matrix interferences. It should be taken into account that direct analysis of the local tap water (Kgs. Lyngby, Denmark), the source of which is an underground reservoir, using μSI-BI-LOV with ion-exchangers or chelating resins was previously proven to be cumbersome for trace element determination [43] owing to the high content of calcium – namely, 128 mg L^{-1} – which corresponds to a hardness of 18 °.

For SRM 1640, a concentration of 30.0±1.2 μg L^{-1} of Ni(II) was determined, which is in good agreement with the certified value (27.4±0.8 μg L^{-1}). A statistical *t*-test was performed to compare the means of the experimental result with the certified reference value [44]. No significant differences were found to be present at a confidence level of 95 %.

Conclusion

A lipophilic/hydrophilic copolymeric sorbent [poly(divinylbenzene-co-N-vinylpyrrolidone)] has been proposed, for the first time, as a sorptive material for selective trace element separation and preconcentration in complex environmental samples (hard waters and brines) utilizing a miniaturized and automated sequential injection LOV system in a bead-injection mode. Hyphenated with detection by ETAAS, the developed flow assembly has proven to be suitable for monitoring trace concentrations of Ni following on-line derivatization with DMG and collection of the organic chelate on the bead surfaces. Compared with hydrophilic sorbents, such as chelating Sepharose and Sephadex ion-exchangers, which have been used as renewable entities in the LOV unit, the co-polymeric material features an improved selectivity in the presence of alkaline earth metals as a consequence of its hydrophobic nature. Thus, in our particular case, ratios of Ca(II) or Mg (II) to Ni(II) as high as 1×10^6 might be tolerated. Furthermore, as opposed to conventional hydrophobic sorbents (e.g. polytetrafluoroethylene and octadecyl chemically modified silicagel), the polymeric beads entail a trouble-free automatic handling in the SI-LOV microconduits due to its hydrophilic/lipophilic balance.

The implementation of new materials as a preconcentration medium in LOV may thus expand the scope of applicability of the third generation of FI to biological and environmental matrices of increased complexity with no need for any ancillary off-line sample pre-treatment procedure.

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Paper VIII

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Hyphenating Multisyringe Flow Injection Lab-on-Valve Analysis with Atomic Fluorescence Spectrometry for On-Line Bead Injection Preconcentration and Determination of Trace Levels of Hydride-Forming Elements in Environmental Samples

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In this work the third generation of flow injection analysis, that is, the so-called micro-lab-on-valve (LOV) approach, is proposed for the first time for the separation, preconcentration, and monitoring of metalloids as hyphenated with atomic fluorescence spectrometry (AFS). This was made feasible by interfacing the micromachined LOV-module with AFS by a multisyringe flowing stream network for on-line postcolumn derivatization of the eluate aimed at generation of hydride species. The potential of this new hyphenated technique for environmental assays was ascertained via determination of ultratrace level concentrations of total inorganic arsenic in freshwater. Employing quantitative preoxidation of As(III) to As(V) in the samples by means of permanganate, the method involves preconcentration of arsenate at pH 10 on a renewable anion exchanger, namely, Q-Sepharose, packed in a LOV microcolumn. The analyte species is afterward stripped out and concurrently prereduced by a 300 μL eluent plug containing 6 mol L^{-1} HCl and 10% KI. The eluate is downstream merged with a metered volume of sodium tetrahydroborate (0.3% w/v) for generation of arsine, which is subsequently quantified by AFS. The flow system facilitates on-column reduction of the retained arsenic with no need for application of programmable stopped flow. Yet, the high concentration of reductant and extreme pH conditions for elution hinder the sorbent to be reused due to gradual deactivation of the functional moieties, so that maximum benefit can be taken from the application of the bead disposal strategy. The proposed procedure is characterized by a high tolerance to metal species and interfering hydride-forming elements. In fact, ratios of Se(IV) to As $\leq$ 5000 and Sb(V) to As $\leq$ 500 are tolerated at the 10% interference level. Under the optimized experimental conditions, a detection limit (3σ) of 0.02 ng mL^{-1} As, a dynamic linear range of 0.05−2.0 ng mL^{-1} As (by tailoring the AFS gain), an enrichment factor of 8.8 for arsenate, and a precision better than 6.0% at the 0.1 ng mL^{-1} level were obtained for the bead-injection mode whenever the loading sample volume was affixed at 3.0 mL. The reliability and accuracy of the automated procedure was ascertained by determining total inorganic arsenic in both spiked environmental waters and certified reference materials of variable matrix complexity (TMDA-54.3 and ERM-CA010) at the low ng mL^{-1} level. No significant differences were found between the experimental results and the certified values at a significance level of 0.05.

As an elemental-specific detector, atomic fluorescence spectrometry (AFS) features improved performance over atomic absorption spectrometry (AAS) regarding minimization of light-scattering effects and background matrix interferences[1] and yielding, in hyphenation with hydride generation (HG) systems, better detection limits for hydride-forming species.[2]

Direct analysis of raw environmental samples via HG-AFS has, however, proven rather cumbersome owing to the ultralow concentration levels of metalloids present in the samples—typically in the sub-ng/mL level—and the concomitant existence of transition-metal ions that might interfere in determination of the evolved volatile compounds. Therefore, ancillary sample pretreatment procedures aiming at removing interfering sample constituents and improving analyte detectability by preconcentration schemes are often imperative for reliable measurements in the environmental field. Actually, maximum benefit in terms of automation, miniaturization, and straightforward sample handling can be earned by application of the first two generations of flow injection.[3−6]

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The third generation of flow injection, the so-called micro-sequential injection lab-on-valve (μSI-LOV),[7,8] has opened up new avenues in chemical analysis regarding sample processing. As the name implies, it works as an integrated laboratory mounted atop a selection valve that allows a multitude of fluidic operations to be executed in an on-line fashion, including sample loading, reagent addition and mixing, and in-valve detection. This miniaturized unit is operated with microliter levels of sample and reagents, thereby downscaling reagent-based assays with the consequent advantages on low reagent consumption and minimized production of chemical waste. In addition, the LOV unit can be configured as a jet-ring-cell to execute sorptive extraction procedures with renewable surfaces (the so-called bead-injection analysis)[9] for analyte isolation/preconcentration prior to presentation to the detector.

Although the multipurpose micromachined structure has been exploited for microfluidic operations as a front end to modern analytical instrumentation including capillary electrophoresis,[10,11] inductively coupled plasma mass spectrometry,[12] liquid chromatography,[13] electrothermal atomic absorption spectrometry,[14,15] cold-vapor atomic spectrometry,[16,17] and electrospray mass spectrometry,[18,19] the marriage between LOV-BI and HG-AFS for assays of trace-level metalloids has not been reported to date. The reason lies in the hindrance of implementing post-LOV derivatization reactions into the discontinuously operating μSI systems, as demanded in on-line BI-HG schemes, because of the requirement of having to aspirate all solutions in SI via a holding coil. In fact, as recently described, the coupling of μSI-BI with cold vapor generation demands utilization of a secondary sequential injection setup for appropriate system performance.[17] Therefore, combination of LOV with BI has been so far mostly applied to on-column spectroscopy[20−22] where the beads are trapped within an in-valve flow cell and continuously monitored by UV−vis spectroscopy or spectrofluorometry or to atomic spectrometry measurements based on injection of the analyte-containing eluate directly into the detector without further processing.[12,14,15]

The recently introduced multisyringe flow injection (MSFI) analysis approach,[23−25] combining the advantages of multichannel operation with the use of a suite of syringes of variable volume, which ensure a constant, pulseless flow as well as accurate metering of microvolumes of solutions via multicommutation protocols, should be viewed as an appealing alternative to SI for accommodation of LOV methods.

The aim of this manuscript is therefore to explore the performance and potential applicability of the hyphenation of BI-LOV with MSFI prior to AFS detection for automatic on-line sample treatment by renewable solid-phase extraction and subsequent derivatization of the eluate for monitoring of trace levels of hydride-forming species. Determination of total inorganic arsenic was selected as a target measurand in order to demonstrate the analytical potential of such a hyphenation.

Arsenic is ubiquitous in the environment due to natural sources and widespread anthropogenic use as a pesticide and herbicide, growth promoter for swines, food additive to combat deceases in poultry, as well as preservative for wooden structures, among others. Inorganic arsenic, including arsenite and arsenate, dominates in freshwater with concentrations of various orders of magnitude higher than those of organic forms.[26] The toxicity of arsenic strongly depends on its chemical forms, inorganic arsenic species being more toxic than the organic ones.[27] According to the accumulation of evidence for the chronic toxic effects of inorganic arsenic,[26,27] the regulatory limit in drinking water, as expressed by the World Health Organization (WHO), the U.S. Environmental Protection Agency (US-EPA), and the European Community (EC), is set at 10 μg L^{-1}.[28]

Increased awareness of the presence of toxic inorganic arsenic in the environment and the need for routinely determining its low concentrations in natural waters have, thus, made development of ultrahighly sensitive, automated, and affordable methods imperative, and these requirements can readily be effected by performing bead injection-LOV analysis in combination with MSFI-HG-AFS.

EXPERIMENTAL SECTION

Instrumentation. A multisyringe piston pump with programmable speed (MicroBU 2030, Crison, Alella, Spain) equipped with four high-precision bidirectional syringes (S_1, S_2, S_3, S_4) (Hamilton, Switzerland) connected in block to a single stepper motor was utilized as a multiple liquid driver. S_1 and S_2 with a capacity of 5.0 mL contained the carrier (ammonium/ammonia buffer) and Milli-Q water, respectively. S_3 and S_4 with a capacity of 2.5 mL served for rinsing the sampling line between consecutive samples or standards and providing the hydride generation reagent, respectively. Three-way solenoid valves (V_1, V_2, V_3, V_4) (N-Research, Caldwell, NJ) were mounted atop of each syringe, enabling communication with the liquid reservoirs in the OFF position or with the flow manifold whenever activated to ON. The MSFI-LOV assembly hyphenated to hydride generation-atomic fluorescence spectrometry is schematically illustrated in Figure 1.

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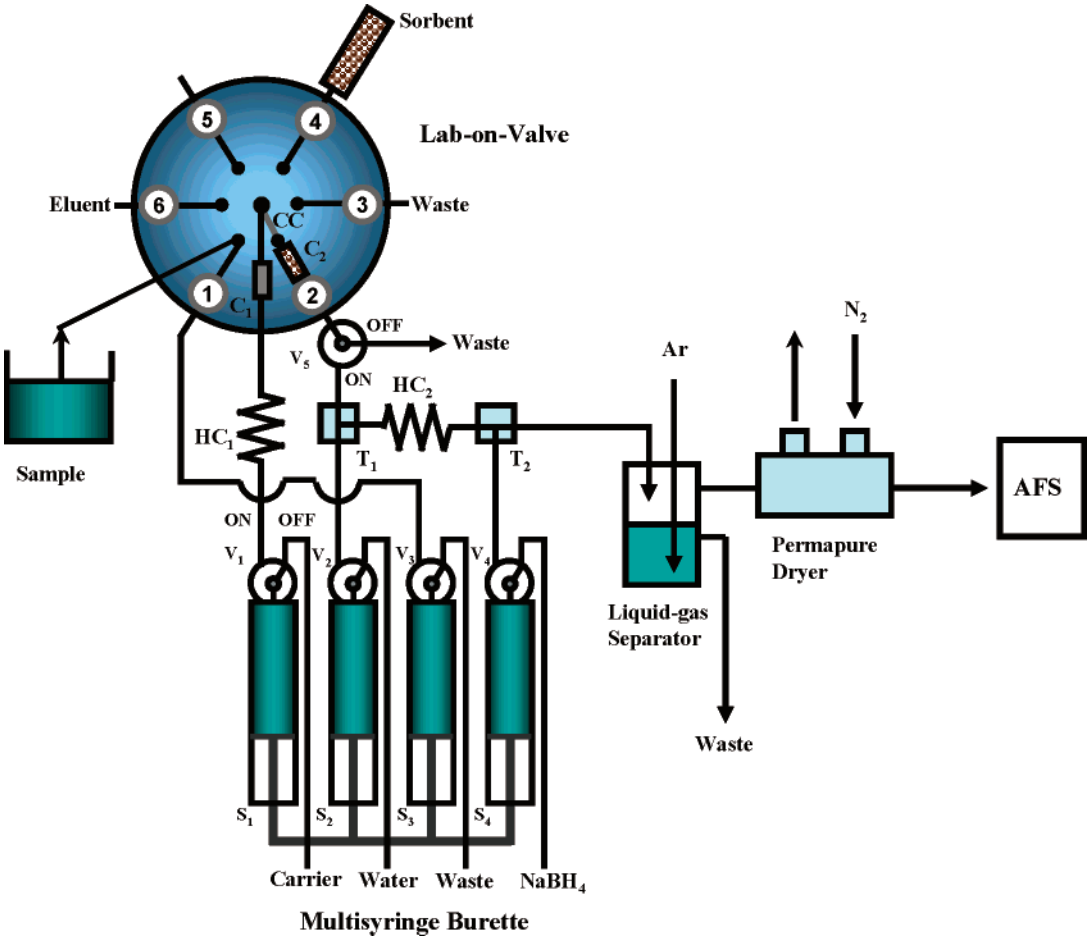

Figure 1. Schematic illustration of the Multisyringe flow injection-lab-on-valve manifold hyphenated to hydride generation−atomic fluorescence spectrometry for the bead-injection preconcentration and determination of trace-level concentrations of total inorganic arsenic. Carrier, 0.01 mol L^{-1} ammonium chloride/ammonia buffer at pH 10 + 8 × 10^{-5} mol L^{-1} citrate; $NaBH_4$, 0.3% (w/v); eluent, 10% KI + 6 mol L^{-1} HCl + 0.2% ascorbic acid; S_1–S_4, syringes; V_1–V_5, solenoid valves; T_1 and T_2, three-way connectors; HC_1 and HC_2, holding coils; C1 and C2, LOV microcolumn positions; CC, central communication channel; AFS, atomic fluorescence spectrometer.

The flow network was built from PTFE tubing of 0.8 mm i.d., except the 127-cm long postcolumn holding coil (HC_2), which was made from 1.0 mm i.d. PTFE tubing, and the 282-cm long holding coil (HC_1), interfacing the liquid driver with the LOV microconduits, and the 20-cm long line connecting HC_2 with the gas−liquid separator, which were all made from 1.5 mm i.d. PTFE tube. A discrete solenoid valve (V_5) was implemented at the outlet of the LOV microcolumn to solely deliver the solution from the miniaturized module into the MSFI flow network at the precise instant for on-line derivatization. The manifold also contains two Teflon three-way T pieces (T_1 and T_2) for connection of the various lines.

The LOV microconduit, fabricated from hard PVC and encompassing six integrated microchannels (1.66 mm i.d./12.0 mm length), was mounted atop a six-port multiposition selection valve (SV, Valco Instruments, Houston, TX). The central port of the integrated LOV sample processing unit, connected to S_1, is made to address the peripheral ports of the unit (1−6) for sequential aspiration of the various constituents for the BI process via the central communication channel (CC) in the SV. Two of the LOV channels (the central one and that of port 2) served as microcolumn positions for the renewable beads. To contain the sorbent within the cavities of the LOV microbore module and prevent them from escaping, the outlets of the columns were provided with small pieces of rigid PEEK tubing of 1.60 mm o.d. (Upchurch Scientific, Oak Harbor, WA) working as stoppers. The diameter of the rods was slightly smaller than that of the LOV conduits, thereby allowing the liquid to flow freely along the walls but effectively entrapping the sorptive material. The bead container and eluent reservoir were attached to the peripheral ports 4 and 6, respectively, while port 3 was used for sorbent disposal after each analytical assay. The specially designed dual channel (port 1) is utilized for sample introduction into the flow system, the outgoing channel being connected to S_3, thereby permitting a thorough rinsing of the sampling tubing between samples to avoid analyte carryover effects.

A modified Vijan-type U-tube gas−liquid separator (GLS, H003G102 “B” type, P.S. Analytical Ltd, U.K.) was used for isolation of the on-line evolved hydrides. Argon was selected as the inert gas to assist the efficient separation and transport of the gaseous analyte into the detector. To prevent entrainment of the moisture of the gaseous phase into the detector and subsequent deleterious effects in the readouts, a shell-and-tube configured drying membrane (Perma Pure Inc, Toms River, NJ) utilizing nitrogen as a purge gas was coupled to the outlet of the GLS.

The generated arsine was detected via a PSA-10.044 Excalibur Atomic Fluorescence Spectrometer (AFS, P. S. Analytical Ltd.,

Table 1. Operating Procedure of the MSFI-LOV Analyzer Hyphenated to HG-AFS for On-Line Preconcentration and Determination of Inorganic Arsenic in Environmental Waters

			position of solenoid valves[a]							
step	description	operation (liquid driver)	V1	V2	V3	V4	V5	LOV position	flow rate (mL min^{-1})	volume (μL)
1	rinsing of manifold lines	dispense	on	off	off	off	on	2	10	1820
2	bead loading	aspirate	on	off	off	off	off	4	1	20
	cleansing of sorbent	dispense	on	off	off	off	off	2	2	2000
3	time-based injection of sample into HC_1	aspirate	on	off	off	off	off	1	5	3000
	analyte loading plus sample cleanup	dispense	on	off	off	off	off	2	3	3500
4	loading of eluent into HC_1	aspirate	on	off	off	off	off	6	3	300
	analyte elution/prereduction	dispense	on	off	off	off	on	2	1	1000
5	filling-up of syringes	aspirate	off	off	off	off	off		10	5000
	postcolumn derivatization	dispense	off	on	off	on	off		18	1500 (for S2)
	transportation of arsine into GLS and recording of AFS transient signals	dispense	off	on	off	off	off		12	3500
6	filling-up of syringes	aspirate	off	off	off	off	off		10	4500
	removal of sorbent from C_2	aspirate	on	off	off	off	off	2	3	500
	discarding of beads to waste	dispense	on	off	off	off	off	3	5	1500

[a] On: Connection of the syringe(s) with the flow system. Off: Connection of the syringe(s) with the external reservoir(s).

U.K.) equipped with an arsenic-boosted discharged hollow cathode lamp (Photron Pty. Ltd., Australia) operating at a primary current of 27.5 mA and a boost current of 35 mA. The analytical signals were integrated in the peak height mode. An external cylinder of hydrogen was needed to support the flame of the AFS. The flow rates of argon, hydrogen, and nitrogen for HG-AFS measurements were affixed to 250, 55, and 1500 mL min^{-1}, as reported elsewhere.[29]

The operational procedures for the MSFI-LOV analyzer were computer controlled by the software package AutoAnalysis (Sciware, Spain) 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, selection valve, and atomic fluorescence spectrometer.

Reagents and Samples. All chemicals were of analytical-reagent grade, and doubly deionized water (18.2 MΩ cm) obtained from a Millipore system (Milllipore Synthesis A10, France) was used throughout for solution preparation. All glassware was soaked in a 10% (v/v) nitric acid solution and afterward cleansed with Milli-Q water.

Arsenic(III) and arsenic(V) stock solutions were prepared by dissolving 1.320 g of As_2O_3 (Merck) and 1.534 g of As_2O_5 (Aldrich) in 1000 mL of 0.1 M NaOH solution and stored at 4 °C. The prereductive eluent containing10% (m/v) KI (Fluka), 6 M HCl (Riedel-de Haën), and 0.2% (m/v) ascorbic acid (Fluka) was prepared daily. The role of ascorbic acid is to prevent oxidation of iodide to free iodine by dissolved oxygen. A 4.0% (m/v) $NaBH_4$ (Merck) stock solution was prepared weekly by dissolving of the appropriate amount of salt in 0.2 mol L^{-1} NaOH. The 0.3% (m/v) $NaBH_4$ working solution was prepared daily by dilution of the stock solution in Millipore water. A 0.01 mol L^{-1} ammonium chloride/ammonia buffer (pH 10) was used as a carrier solution in S_1. Working standard solutions of arsenate (0.05−2.0 μg L^{-1}) were prepared daily by stepwise dilution of the above-mentioned stock solution in the ammonia-ammonium buffer.

The Q-Sepharose Fast Flow strong anion exchanger (90 μm average particle size, Amersham Biosciences, Sweden), consisting of cross-linked 6% agarose furnished with diethyl-(2-hydroxypropyl)aminoethyl moieties, was used directly in the LOV with no need for any additional preconditioning protocol. The beads were contained in a dedicated syringe reservoir mounted upward on port 4 as a 20% ethanol suspension. The tip of the syringe is equipped with a short PEEK tubing piece of 0.76 mm i.d. (Upchurch), which fits via a gasket into the machined LOV.

Environmental waters were filtered through 0.45 μm cellulose esters membrane filters (MF-Millipore) immediately after collection. Total inorganic arsenic was determined by adding 2 × 10^{-6} mol L^{-1} potassium permanganate and 8 × 10^{-5} mol L^{-1} sodium citrate to the samples prior to buffering at pH 10. The standard reference materials, viz., ERM-CA010 (Trace Elements in Hard Drinking Water) and TMDA-54.3 (Trace Element Fortified Lake Ontario Water), were from LGC-Promochem in the United Kingdom and the National Laboratory for Environmental Testing in Canada and subjected to a pretreatment like the environmental waters.

Analytical Procedure. The operational sequence for sorptive preconcentration of inorganic arsenic onto the LOV renewable microcolumns and further on-line HG-AFS detection exploiting MSFI in a multicommutation fashion is compiled in Table 1, where details of the LOV positions and operation of the multisyringe pump are given along with the corresponding consumption of sample and reagents and delivery flow rates. The overall sample processing cycle involves six steps, viz, system preconditioning, microcolumn packing, analyte collection, elution/prereduction of preconcentrated species, hydride transfer reaction and transportation into the AFS, and finally sorbent disposal, summarized as follows.

(1) System Preconditioning. Initially, syringes S_1, S_2, and S_4 and the corresponding tubing connecting the liquid drivers with the HC_1 and mixing points T_1 and T_2 are cleansed and the syringes filled with carrier, Milli-Q water, and sodium tetrahydroborate, respectively. On changing the sample, S_3 is set to aspirate a well-defined volume of sample solution (namely, 200 μL) past flow-

(29) Semenova, N. V.; Leal, L. O.; Forteza, R.; Cerdà, V. *Anal. Chim. Acta.* **2002**, *455*, 277−285.

through port 1 to rinse the sampling line. S_1 is afterward programmed to deliver 1820 μL of carrier to the MSFI manifold for cleansing of the postcolumn derivatization flow network.

(2) Microcolumn Packing. A minute, metered volume of the anion-exchanger suspension (namely, 20 μL) is aspirated slowly into the microcolumn cavity C_1. The communication channel (CC) is then connected to the peripheral port 2 to transfer the beads to the C_2 position and rinse the surfaces with the buffer solution.

(3) Sample Loading. S_1 is set to draw a 3000 μL sample in a time-based injection mode from the container into HC_1, whereupon the flow is reversed and the sample segment plus 500 μL of buffer are pumped to C_2 for effecting the preconcentration of arsenate and removal of weakly or nonretained matrix constituents from the bead surfaces. It should be noted that during sample loading the solenoid valve V5 is switched to Off to prevent introduction of potential sample matrix interferents into the flow network

(4) Analyte Elution/Prereduction. To this end, a 300 μL eluent plug is aspirated from port 6 and dispensed forward to C_2 for quantitative stripping of the collected arsenate with concomitant on-column reduction to arsenite for the forthcoming generation of the volatile species, the eluate being directed into holding coil HC_2.

(5) Hydride Generation/Detection. The postcolumn derivatization reaction is performed by merging of the prereduced eluate plug downstream with a metered volume of sodium tetrahydroborate, the stacked zones being delivered to the gas−liquid separator for completion of the reaction and isolation of the evolved arsine, which is guided into the flame of the AFS via a stream of Ar carrier gas.

(6) Sorbent Disposal. The beads packed within the LOV microcolumn are readily discarded on-line (via port 3) after being moistened with carrier solution. Hence, the MSFI-LOV-AFS setup is ready to initiate a new analysis cycle with a fresh portion of beads, thus overcoming deterioration of the analytical performance of the hyphenated analyzer due to the progressive deactivation of the sorbent material.

RESULTS AND DISCUSSION

Configuration of the MSFI-BI-LOV System Coupled to HG-AFS for Determination of Trace Levels of Inorganic Arsenic. The actual concentration of the various chemical forms of inorganic arsenic in waters is strongly affected by the pH as a consequence of the difference of the acid dissociation constants of arsenic(III) (pK_{a1} = 9.1, pK_{a2} = 12.1, and pK_{a2} = 13.4) and arsenic(V) (pK_{a1} = 2.1, pK_{a2} = 6.7, and pK_{a3} = 11.2).[30] In marine/natural waters arsenate predominantly exists as oxoanionic forms like monohydrogenarsenate and dihidrogenarsenate and arsenite as a neutral form, that is, as arsenous acid.[31] An alkaline milieu would therefore benefit formation of anionic species that can be on-line preconcentrated on strong anion exchangers (e.g., ASTM-Merck, IONAC Na-38, Muromac 2 × 8, Amberlite IRA-410) as reported in the literature using permanently packed columns.[32−34]

However, some of the above materials do not fulfill the stringent requirements for being used as renewable surfaces in the LOV microconduits. Actually, the beads must (a) be perfectly spherical (i.e., in the form of globe-shaped particles), (b) be uniform in size distribution (falling within a range of 40−150 μm), (c) be unaffected by pH over a wide range, and (d) have a high specific surface or exchangeable capacity, and (e) have a density close to that of water. To this end, the commercially available strong anion-exchange Q-Sepharose resin, which has proven to be highly efficient for collection of ionic species in LOV,[35] has been selected here as a disposable sorptive material.

The prevailing procedure for chemical hydride generation (HG) of metalloids involves reaction of the target compounds with tetrahydroborate in acidic medium to produce gaseous hydrides.[36,37] The reaction is pH dependent in which the tetrahydroborate initially reacts with acid to produce nascent hydrogen which then reacts with the analyte to form the respective hydride. When coupling on-line anion-exchange preconcentration with HG, one possible approach is to retain temporally both the reducing agent and the analyte on the sorbent material in either a concurrent or a sequential fashion, followed by passage of an acidic eluent for on-column generation of the hydride. It has been found that this procedure minimizes matrix interferences as compared with those encountered when applying homogeneous reactions in solution.[38] Detection limits are also improved as a result of the decrease in blank signals since a smaller amount of reagent is needed, and reagent contaminants are removed by the immobilization process, producing a purer reagent.[38,39]

Accordingly, preliminary experiments involving the consecutive loading of alkaline tetrahydroborate and arsenite-containing solution onto the LOV microcolumn with further acidic reductive elution were conducted. Yet, the analytical sensitivity of the LOV-BI system did not suffice for determination of trace levels of metalloids. This discrepancy with earlier researchers is attributed to the minute dimensions of the renewable packed column which gives rise to a lower on-column reagent concentration. In addition, column breakthrough was observed at the very early stage of reagent loading as a consequence of its monovalent nature.

Therefore, a postcolumn eluate treatment modality was adopted, thus exploiting the versatility of the LOV-MSFI coupling for microfluidic operations. Hence, a well-defined plug of the hydride-forming reagent was added to the eluate zone in a multicommuted merging zones mode prior to gas−liquid separation. It should be stressed, however, that while elution of sorbed arsenic requires moderately low flow rates (≤1.0 mL min^{-1}), formation of gaseous hydrides shows extremely fast kinetics[29] and delivery rates higher than 10 mL min^{-1} are needed to facilitate evolvement of the volatile species within the gas−liquid separator, thus rendering improved analytical signals. Despite the divergent kinetics and, hence, different hydrodynamic demands, both reactions can be performed on-line consecutively by appropriate arrangement of the MSFI network and making full use of programmable flow. To this end,

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an ancillary three-way solenoid valve was located at the outlet of the sorbent to detach the bead-injection protocol from the postcolumn derivatization reaction, so that optimal conditions for both sample processing and analyte detection are ensured. The multisyringe flow manifold also capitalizes on the availability of simultaneously operating liquid drivers, whereby delivery rates as high as 20 mL min^{-1} can be used in the post-LOV flow network without appreciable increase of flow impedance.

Sample Loading. Bearing in mind the generation of gaseous arsine following the sorptive preconcentration of the target species, it might be assumed that inorganic arsenic should be collected on the sorbent in the form of arsenite for appropriate system performance. Although it has been reported in the literature that reusable packed-bed anion-exchange reactors are efficient media for uptake of As(III),[32,33] arsenite breakthrough was, independently of the loading flow rate, detected in the minute LOV column, which is in agreement with our previous results (see above) regarding immobilization of tetrahydroborate on the anion-exchange beads. Actually, a mere 40% of the loaded analyte from a 0.75 μg L^{-1} As(III)-containing standard plug was collected on-line, even in strong alkaline milieus (pH $\geq$ 12), due to the single charge of the oxoanion. On the other hand, As(V) exists in solution predominantly as multicharged species at pH $\geq$ 7.0, which might facilitate the improved uptake by the ion-exchange processes. Preliminary results conducted under identical conditions as detailed for As(III) revealed that As(V) can be retained on the LOV packing material with efficiencies > 85%. Therefore, it is readily feasible to improve the retention efficiency and thus, in turn, the analyte detectability by quantitative conversion of all inorganic arsenic forms into the highest oxidation state followed by pH adjustment. To this end, potassium permanganate was employed as an oxidation agent, which has been demonstrated to oxidize As(III) into As(V) almost instantaneously at near-neutral conditions.[34] The concentration of permanganate was kept at 2×10^{-6} mol L^{-1} to prevent undesired pre-elution of collected arsenate.

The effect of sample pH on the analytical sensitivity was investigated over the pH range 6−12 using a 0.2 μg L^{-1} As(V)-containing standard solution. The fluorescence intensity increased sharply within the pH range 6−9 because of the generation of bicharged anionic forms, while a more gradual increase was detected at pH 9.5 and onward. For efficient preconcentration of inorganic arsenic, while minimizing the hydrolysis of potentially coexistenting metal ions, the sample was buffered at pH 10 using 0.01 mol L^{-1} ammonium chloride/ammonia to which 8×10^{-5} mol L^{-1} citrate was added as a masking agent for hindering formation of insoluble metal oxyhydroxides.

As to the sample loading rate for the integrated LOV microcolumn, flow rates ranging from 0.5 to 3.0 mL min^{-1} were assayed taking into account the intrinsic feature of highly cross-linked Sepharose beads for enduring high solution flow rates with negligible squeezing and efficient uptake of charged species.[14,35,40] As expected, no significant differences were found for the overall column loading flow rates, yet delivering rates > 3.0 mL min^{-1} should be avoided because any increase of flow resistance might affect the long-term operational performance of the solenoid valves of the flow network.

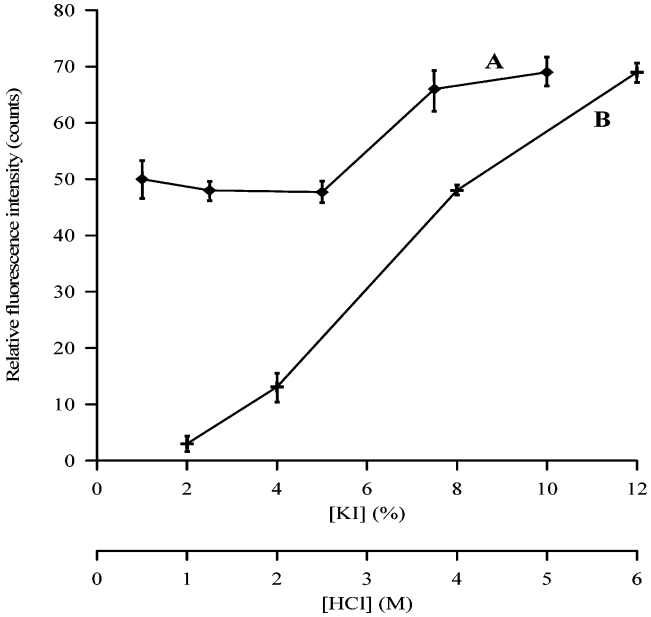

Figure 2. Effect of the composition of the combined prereductive eluent on the MSFI-LOV-AFS readout. (A) Concentration of potassium iodide. (B) Concentration of hydrochloric acid. Standard concentration: 0.2 ng mL^{-1} As(V). Sample loading volume: 3 mL. Eluent volume: 0.3 mL. Hydride-forming reagent: 0.3% $NaBH_4$. See text for further details.

On-Column Prereduction and Hydride Generation. After isolation of inorganic arsenic from the matrix constituents by ion-exchange preconcentration on the sorptive material, the target oxoanion is subjected to two operations prior to final quantitation by HG-AFS. First, the collected arsenate should be stripped out from the LOV microcolumn by application of either a sharpened increase of the ionic strength of the mobile phase or a pH gradient through the anion exchanger. Second, the arsenate should be derivatized into a gaseous hydride of arsenic upon reaction with a reducing agent. Although direct evolvement of arsine from arsenate has been reported in the literature using high concentrations of sodium tetrahydroborate,[33,41] the sensitivity of on-line measurements is frequently impaired as a consequence of the low reduction rates,[42,43] and detection limits are, additionally, deteriorated owing to the increase of blank signals.[38] Thus, a prereduction step of arsenate to arsenite prior to arsine generation is often indispensable in flow systems.[44,45]

Taking into account the above considerations, a combined reagent involving potassium iodide in acidic medium was selected as a prereductive eluent for removal of As(V) from the LOV packing with concomitant on-column derivatization. Various concentrations of reductant varying from 1.0% to 10% (w/v) KI in 5.0 mol L^{-1} HCl were assayed. Higher concentrations rendered oversaturated solutions at such highly acidic conditions. According to the results shown in Figure 2A, the MSFI readouts increased sharply from concentrations of 5.0% and onward. Whenever the

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concentration of prereductant was affixed to the maximum allowed concentration, i.e., 10%, the higher the acidity the better the analytical sensitivity (see Figure 2B), yet, concentrations > 6.0 mol L^{-1} HCl were inappropriate due to lack of quantitative dissolution of the KI. Therefore, an acid prereductant solution containing 10% KI in 6 mol L^{-1} HCl was selected as an eluent for the LOV-BI-MSFI system. The acidic medium ensures protonation of the target anionic species, making the neutral substances inaccessible for the quaternary ammonium moieties of the sorbent, while the potassium iodide, on one hand, increases the eluting strength of the reagent and, on the other hand, fosters prereduction of collected arsenate. The acidic medium also facilitates conversion of As(V) into As(III) as strong acidic conditions are imperative for efficient reduction by potassium iodide.[45] In contrast to earlier work involving reaction in the homogeneous phase,[44−46] quantitative reduction of As(V) was accomplished on-line utilizing the above reagent at room temperature with no requirement for incubation of the reactant zone via a stopped-flow approach. This is attributed to the on-column fluidized-bed-like conditions occurring in the LOV microchannels that enhance mixing between the reductant and the enriched zone of arsenate. A further asset of this reductive elution protocol is avoidance of undesired postcolumn dilution of the eluate plug.

The dependence of the volume and flow rate of the combined eluent on the recovery of As(V) from the microcolumn was also evaluated in an automated programmable flow mode. Experimental results revealed that stripping of arsenate was effectively accomplished for eluent volumes ≥ 300 μL. Actually, the recovery factor, defined as the product of the retention efficiency and the elution yield, was calculated to be 94% for the hyphenated setup. A crucial hydrodynamic variable for appropriate system's performance was the elution/prereduction flow rate. In fact, the analytical sensitivity improved by 20% when the eluent flow rate decreased from 3.0 to 1.0 mL min^{-1}. Therefore, a 0.3 mL eluent zone delivered at 1.0 mL min^{-1} was adopted for further investigations.

In order to conduct the postcolumn reaction under optimal experimental conditions for HG, the prereduced eluate was delivered downstream at 18 mL min^{-1} in lieu of the 1.0 mL min^{-1} capitalizing on the discontinuous flow nature of the MSFI and the flexibility of the approach for providing variable flow rates at will. Concerning the concentration of the hydride-forming agent, it was encountered that both the analytical and blank signals increased when the concentration of sodium tetrahydroborate was increased from 0.05% to 0.40% (w/v) (see Figure 3). A 0.3% (w/v) $NaBH_4$ solution, which yielded the best As/blank signal ratio as well as an acceptable absolute blank value (If ≤ 30), was finally selected for on-line As(III) derivatization.

The chemical composition of the carrier solution supplied by S2 was also ascertained. To this end, different concentrations of hydrochloric acid within the 0.1−8.0 mol L^{-1} range as well as Milli-Q water were utilized as carrier streams. No significant differences at the 0.05 level were found between the various transient readouts recorded. This result confirms that the acidity of the eluent suffices for the overall aims of elution, prereduction, and hydride generation.

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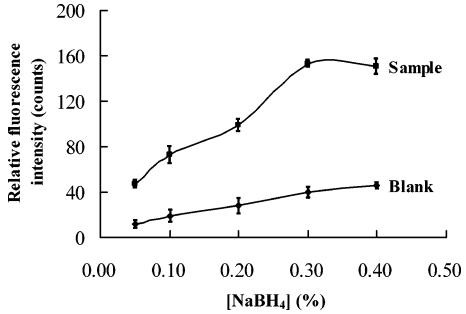

Figure 3. Influence of the concentration of reducing reagent on the signal to blank ratios. Standard solution: 0.2 ng mL^{-1} As(V). Sample loading volume: 3 mL. Eluent: 10% KI + 6 mol L^{-1} HCl + 0.2% ascorbic acid. Eluent volume: 0.3 mL. Volume of reducing reagent: 0.75 mL.

Table 2. Optimal Operational Parameters and Analytical Performance of the MSFI-LOV-HG-AFS System for the Determination of Total Inorganic Arsenic

parameters	value
sample volume (mL)	3.0
loading rate (mL min^{-1})	3.0
eluent/prereductive reagent concentration	10% KI + 6 M HCl + 0.2% ascorbic acid
eluent/prereductive reagent consumption (mL)	0.3
elution flow rate (mL min^{-1})	1.0
$NaBH_4$ concentration (%)	0.3
$NaBH_4$ consumption (mL)	0.75
sorbent amount (mg)	5.0 ± 0.5
regression equation	IF = 359.27 [As, ng mL^{-1}] + 24.93
correlation coefficient	0.9976
linear range (ng mL^{-1})	0.05−0.3 (for an 800-fold AFS gain)
detection limit (ng mL^{-1}, $n = 9$, $3\sigma_{blank}$)	0.02
R.S.D. (%, 0.1 ng mL^{-1}, $n = 7$)	5.7
enrichment factor	8.8
sample throughput (h^{-1})	9.0
concentration factor (h^{-1})	79.2

Analytical Performance. The analytical figures of merit of the MSFI-LOV hyphenated system obtained under optimized chemical and physical conditions are compiled in Table 2. A six-level calibration plot based on least-squares linear regression algorithms was established at an 800-fold detector gain by preconcentrating 3.0 mL of arsenate standards with concentrations ranging from 0.05 to 0.3 ng mL^{-1}. The dynamic range might be however extended by one decade by decreasing of the AFS gain at will. The detection limit was calculated as the concentration of the target oxoanion, rendering an integrated intensity of fluorescence equal to three times the standard deviation of nine consecutive blank measurements.

The overall reproducibility of the method was expressed as the coefficient of variation of replicate analysis of a 0.1 ng mL^{-1} As(V) standard solution using renewable beads. It was proven that, for this particular application, a single solid-phase reactor can be solely reused for a maximum of eight assays as a consequence of the deterioration of the method's precision, resulting in repeatabilities poorer than 12%. Actually, the gradual darkening of the sorbent material, as visible by the naked eye, is the result of the leakage of the pendent functional moieties of

the sorbent and/or hydrolysis of the polysaccharide matrix itself due to the strong acidity of the eluting medium. Therefore, the principle of bead injection with renewable surfaces should be regarded as an unrivalled approach for such analytical applications requiring aggressive eluting media for quantitative stripping of the target species from sorptive columns.

As compared with earlier on-line HG-AFS methods based on exploitation of the various generations of flow injection analysis,[29,32,45] the present LOV hyphenated setup yields a 5-fold improved analytical sensitivity and enhancement of the detection limit by more than 1 order of magnitude. Actually, the detection limit is limited by trace impurities of the hydride-forming reagent, as shown in Figure 3. Furthermore, the MSFI-LOV approach should be viewed as an environmentally friendly analytical method as a result of the minimum consumption of aggressive chemicals in comparison with batch methods, yet with flow injection analysis systems as well. Actually the amounts of $NaBH_4$ and HCl delivered per assay are both 25-fold reduced compared with the first generation of flow analysis using a commercially available system from PS Analytical Ltd.[29]

Investigation of Interferences. One of the most severe side reactions in evolving of gaseous hydrides of metalloids is that caused by the concomitant presence of transition metals in the sample matrix. Actually, metal ions, particularly Ni, Cu, and Co, are known to react with tetrahydroborate and become reduced to colloidal free metals or metal borides.[47] Both species have proven to be superb catalysts for degrading the hydrides before reaching the detector, while the colloidal particles also are efficient media for adsorption of the volatile compounds.[48] To minimize the influence of such interferences, additional measures including adjustment of medium acidity,[44,49] addition of masking agents,[50] or exploitation of kinetic discrimination[47] have been adopted. Yet, an elegant approach selected here for circumventing the above interfering effects is to collect the target oxoanions onto anion-exchanger resins, thus facilitating isolation of the hydride-forming analytes from positively charged species, such as transition-metal ions.

In order to explore the potential analyte breakthrough as a consequence of the competitive uptake of other negatively charged species by the LOV microcolumn, the maximum tolerated concentration of anions commonly encountered in environmental waters was ascertained using a fixed concentration of 0.2 μg L^{-1} As(V) and variable amounts of foreign species. A given concentration level of a chemical species was regarded as interferent whenever the analytical readout of As(V) was affected by more than 10%. The tolerated interferent/analyte ratios of nitrate, chloride, hydrogen carbonate/carbonate, and sulfate were 5×10^6, 2.5×10^6, 5×10^5, and 5×10^5, respectively. As can be seen, the Q-Sepharose ion exchanger can endure rather high concentrations of monovalent anions such as Cl^- and NO_3^-, which is in accordance with earlier observations.[35] As compared with a recently reported flow injection-HGAAS setup for determination of trace-level concentrations of inorganic arsenic following sorptive ion-exchange preconcentration,[33] the LOV-MSFI hyphenated system yields a 5−10-fold improved tolerance to the overall anionic species assayed.

Table 3. Determination of Trace-Level Concentrations of Inorganic Arsenic in Environmental Waters Exploiting MSFI-LOV-BI in Hyphenation with HG-AFS[a]

sample	added (ng mL^{-1})	found (ng mL^{-1})	recovery (%)
tap water	0	<LOD	
(Valldemossa, Spain)	1.0	0.93 ± 0.05	93
	1.5	1.65 ± 0.05	110
underground water	0	0.23 ± 0.03	
(Palma, Spain)	1.0	1.29 ± 0.03	105
	1.5	1.8 ± 0.1	104

[a] The results are expressed as the mean of three replicates ± SD.

The interfering effect of other hydride-forming elements, such as selenium and antimony, which might compete with arsenic for the binding sites of the ion exchanger as well as for the reducing agent, with the consequent decrease of the yield of the on-line arsine generation reaction, were also evaluated. It should be born in mind that Se(IV), Se(VI), and Sb(V) are present at the sample loading pH as mononegatively or dinegatively charged species, and therefore, they are likely to be partially retained on the sorbent. On the other hand, Sb(III) is expected not to affect the ion-exchange process since the predominant forms at pH 10 are the noncharged antimony hydroxide or metaantimonious acid species.[51] The tolerated ratios for Se(IV), Se(VI), and Sb(V) at the 10% interference level were 5×10^3, 5×10^5, and 5×10^2, respectively. The negligible effect of concentrations of Se(VI) as high as 100 mg L^{-1} on the analytical readouts is attributed to the requirement of the aggressive chemical conditions for reduction of Se(VI) to Se(IV), namely, 50% HCl and temperatures > 70 °C,[53] which are not provided in the MSFI analyzer. Actually, as opposed to previous flowing stream methods for direct determination of arsenic in the liquid phase via HG-AFS,[45] the developed LOV system involving solid-phase extraction of the target analyte features improved tolerance for Sb and Se by 20- and 250-fold, respectively.

Validation of the MSFI-LOV System. Environmental water samples, including tap and underground water, as well as certified reference materials of relative matrix complexity (viz., ERM-CA010 hard drinking water and TMDA-54.3 lake water) were utilized to ascertain the reliability and accuracy of the proposed MSFI-LOV preconcentration method. The recoveries obtained for the environmental waters spiked with arsenate at two different levels below the regulatory limits endorsed by the WHO for As in drinking water (i.e., 10 ng mL^{-1}) are listed in Table 3. For determination of arsenic concentrations ≥1.0 ng mL^{-1}, either the AFS gain was tailored within the range 1−500 or the samples were conveniently diluted. Application of the statistical *t*-paired test[53] for the recovery values rendered a calculated *t* of 0.77, which is below the critical value for four degrees of freedom (viz., 3.18) at the 0.05

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significance level, thus revealing the inexistence of multiplicative (nonspectroscopic) matrix interferences for the analyzed samples.

As to the standard reference materials, total inorganic arsenic concentrations of 47 ± 3 (n = 6) and 51 ± 4 ng mL^{-1} (n = 6) were determined for the TMD-54.3 lake water and ERM-CA010 hard drinking water, respectively, which are in good agreement with the certified values, namely, 45.3 ± 7.3 and 55 ± 5 ng mL^{-1} As, respectively. Statistical t tests were conducted aiming at comparing the means of the experimental results with the certified concentrations.[53] No significant differences were found at a confidence level of 95% for any of the assayed materials since the calculated $|t|$ values were 1.39 and 2.44 for TMD-54.3 and ERM-CA010, respectively, for a critical value of 2.57.

Further research is currently focusing on application of the proposed MSFI-LOV methodology for monitoring trace-level concentrations of total arsenic in foodstuffs, following alkaline enzymatic hydrolysis and on-line sample processing, as an appealing tool for the Key Action of "Food Quality and Safety" within the 6th Framework Programme of the EU. Speciation analysis can readily also be effected exploiting selective retention of As(V) on the microLOV ion exchanger by appropriate pH adjustment.

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