Solid reactors in sequential injection analysis: recent trends in the environmental field

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The second generation of flow-injection analysis (FIA), so-called sequential injection (SI), has already been consolidated as an attractive flowing-stream approach in several analytical fields, with advantages over the first generation of FIA in terms of automation, miniaturization, and sample and reagent consumption. A further noteworthy feature is the inherent versatility of the flow network to implement unit operations at will with no need for manifold re-configuration. The present review discusses the potential of SI to accommodate solid reactors and packed columns in the flow set-up for environmental applications, aiming to facilitate on-line chemical derivatization, chromatographic separation of target species, removal of interfering matrix compounds, or determination of trace levels of analyte via sorptive preconcentration procedures. In this context, the concept of renewable surfaces, so-called SI-bead injection (SI-BI), used in either the jet-ring or lab-on-valve (LOV) configurations, is presented as a front-end to many detectors. This article also outlines recent trends focused on exploiting SI as an automated tool for handling solid samples of environmental concern and accommodating dynamic fractionation schemes for trace elements.
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Keywords: Sequential injection; Solid reactor; Preconcentration; Matrix removal; Chromatographic analysis; On-line soil fractionation

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1. Introduction

Flow techniques are being consolidated as powerful tools for the routine control of parameters in various samples of environmental origin. The widespread acceptance of the first generation of flow injection (FI) for analytical applications is due to its extremely straightforward configuration, easy operation, low costs, and compatibility with almost any detection principle [1–4]. Automated analyses with high sample throughput are attained using relatively simple instrumentation. The micro-format of FI results in considerable reduction of sample and reagent consumption compared to classical wet-chemical methods of analysis [1,2]. In addition, the highly reproducible mixing of streams, controllable dispersion and timing enable novel kinetic applications never thought of before.

The monitoring of metastable intermediate reaction products clearly illustrates the powerful capabilities of FI. The ready adaptation of batch methods permits FI to be used in compliance with many official standard methods (namely, US Environmental Protection Agency (US EPA), International Standards Organization (ISO) and European Norms) [5].

Environmental safety is another attribute of FI. The closed-system chemistry not only decreases contamination of the sample but also prevents operators coming into contact with hazardous chemicals. Moreover, as a consequence of its inherent miniaturization, waste generation, and hence also the costs for waste disposal, are considerably decreased. FI has also attracted the attention of many researchers owing to the ability of fulfilling the current demands in environmental analysis. The fast response of FI makes the analytical information available in real time, which is especially desirable in monitoring schemes. FI has also been exploited for in-field analysis aiming to obtain high-resolution temporal and spatial data without the need for discrete sample collection and storage [6]. This is of great interest whenever off-site analysis or excessive sample handling is unacceptable due to rapid transformation of the target analyte.

Despite the aforementioned benefits of FI with respect to its manual counterparts, the incompatibility of the elastic tubes of peristaltic pumps with concentrated acids or bases and organic solvents usually necessitates periodical recalibration of the

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system or the incorporation of more expensive reagent-resistant tubing. The physical adsorption of organic analytes onto hydrophobic manifold tubing is another practical limitation commonly described. Though performance of multiparametric determinations has been described by several authors [7–9] via appropriate microfluidic handling of the injected sample plug, or the use of a single multidetection system or several detectors arranged in series, the simultaneous monitoring of various environmental parameters has been limited to rather particular applications. It should be also stressed that the continuous flow of solutions, in line with FI philosophy, results in excessive consumption of carrier, reagent and sample during the overall analysis protocol.

Sequential injection (SI) was proposed in 1990 as a new alternative concept to FI for handling solutions [10]. While most FI procedures employ continuous, uni-directional pumping of carrier and reagent streams, SI is based on using programmable, bi-directional, discontinuous flow, which is precisely coordinated and controlled by computer [11,12]. Whereas in conventional FI manifolds the sample volume is inserted into the carrier stream and subsequently merged downstream with reagents, SI is based on the sequential aspiration of precise volumes of sample and reagent(s) into a holding coil (HC). The loaded segments are subsequently propelled forward towards the detector, on their way undergoing dispersion and thereby partial mixing with each other, and hence promoting the chemical reaction, the result of which is monitored by the detection system. A schematic illustration of an SI manifold is presented in Fig. 1.

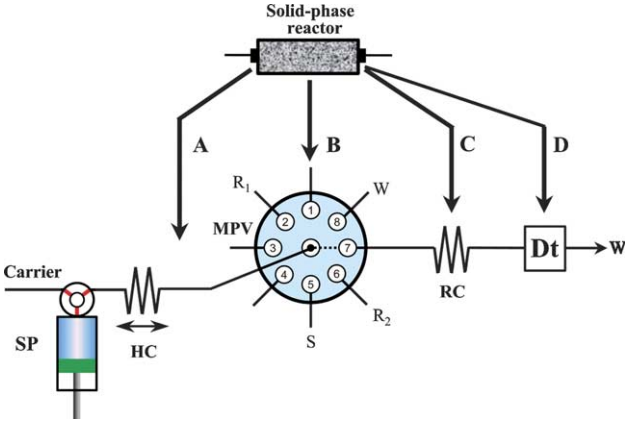

Figure 1. Schematic illustration of the main components of a sequential injection (SI) set-up. The various locations (A–D) of the flow network for the accommodation of the solid-phase reactor are also highlighted. SP, Syringe pump; HC, Holding coil; MPV, Multiposition valve; RC, Reaction coil; S, Sample; R, Reagent; Dt, Detector; W, Waste.

The core elements of the SI network are:

(1) a multi-position valve (MPV) furnished with a central communication channel that can be made to address each of the peripheral ports; and,

(2) a syringe pump (SP) used as liquid driver that allows the manipulation of sample and reagent volumes at the low μl level with high precision, and reproducibly permits flow reversals and exploitation of stopped-flow schemes.

One of the most relevant, distinctive features of SI is its inherent flexibility for accommodating a plethora of unit operations and developing multiparametric determinations. This is facilitated by incorporating additional reactors and modules, either in the flow lines or at the external MPV ports, as detailed in Fig. 1. Thus, SI has proved to be a suitable tool for accommodating and handling solid materials as an integral requirement for the chemical assay, rather than for minimizing the consumption of costly reagents as in FI [13]. Consequently, this flowing-stream approach has opened new ways of performing automated analysis involving solid reactors, as detailed in the following sections with particular emphasis on environmental applications.

2. Solid reagents for on-line derivatization

Like the first generation of FI, SI is conventionally regarded as a liquid-handling technique, whereby solid reagents are dissolved in buffers or aqueous solutions and then attached to the external ports of the MPV. One of the common shortcomings of flow-based analyzers, when intended for long-term monitoring protocols, is the possible chemical instability of the wet reagents. However, this drawback may be overcome in SI set-ups by handling powdered (lyophilized or crystallized) reagents, which can be stored under less stringent conditions, thus allowing *in situ* reagent preparation. The solid reagents are placed in cartridges at the peripheral ports of the MPV (i.e. in position B in Fig. 1), whereas the liquid-reagent medium may be utilized as a carrier or simply connected to another MPV port. The SP is set to dispense a minute, well-defined volume of solvent (typically $\geqslant$ 200 μl) into the reagent cartridge, whereupon a metered volume of dissolved powder is subsequently aspirated into the HC to initiate the derivatization reaction. This sequence is repeated as many times as required according to the scheduled procedure. Dissolution and reagent homogeneity are accomplished by combining forward-backward flow and manipulating air segments within the SI microbore conduits. Passage of the bubbles through the liquid bulk ensures concomitant disruption of the vertical concentration gradient [14].

The above operational approach is not restricted to *in situ* reagent preparation, but is also applicable to other operations, such as on-line analyte derivatization,

indicating the inherent analytical versatility of the SI concept. In fact, the arrangement of reducing/oxidizing columns in parallel at the various MPV ports yields automated systems with multiparametric capabilities, as demonstrated by Galhardo and Massini [15]. These authors assembled an SI analyzer for sequential determination of Fe(III), Fe(II), NO_3^- and NO_2^- in natural and wastewaters using conventional, well-accepted chemistries. The determination of the oxidized species was performed after their reduction in Jones and copperized cadmium columns, respectively, using forward and reverse flow directions. In the latter case, further reduction of nitrite is avoided by precise control of system fluidics. Solid-phase reactors placed in the reaction coil of single-line SI analyzers prior to the detection system (See Fig. 1C and Fig. 2A) facilitate performance of on-line redox reactions in a forward-flow fashion, as demonstrated by Naidoo and Van Staden [16] via the spectrophotometric determination of Mn(II) in environmental waters and effluent streams following oxidation to permanganate in a solid lead(IV) dioxide reactor.

The open architecture of SI can be exploited to its full potential in the third generation of flow-injection analysis, the so-called Lab-on-Valve (LOV) [17,18] that has been designed as a microfluidic device for scaling reagent-based assays down to the sub-microliter level. In addition, solid reactors may be easily accommodated at external LOV positions, as in SI-MPV, for automated redox derivatization, thereby facilitating automated sample processing and integration of multi-elemental assays in the miniaturized unit, as exemplified in the monitoring of nitrate, nitrite and orthophosphate in environmental waters [19].

Although the discussion presented above for on-line sample processing encompassed packed-bed columns with interactive surfaces, non-reactive bead reactors, so-called single-bead-string columns, should be regarded as a promising way to enhance zone overlap between sample and reagent plugs in SI, while ensuring that band broadening is minimal [20,21]. It should also be stressed that sequential, rather than simultaneous, aspiration of solutions inherent to SI typically results in longer residence times for proper interdispersion between segments as a consequence of the predominance of axial dispersion. The incorporation of these inert reactors, involving acid-washed silanized particles, mostly between the MPV and the HC (position A in Fig. 1) has therefore opened new frontiers in developing and accelerating chemical reactions in the microconduits of SI analyzers.

3. Solid materials for chromatographic separations

In the past few years, chromatographic separations at low or moderate pressure have been accomplished by replacing the common HPLC pump with an SI-syringe pump, thereby gaining full benefit of the versatility of the SI analyzer for on-line sample treatment while ensuring automated fluidic control of the entire chromatographic

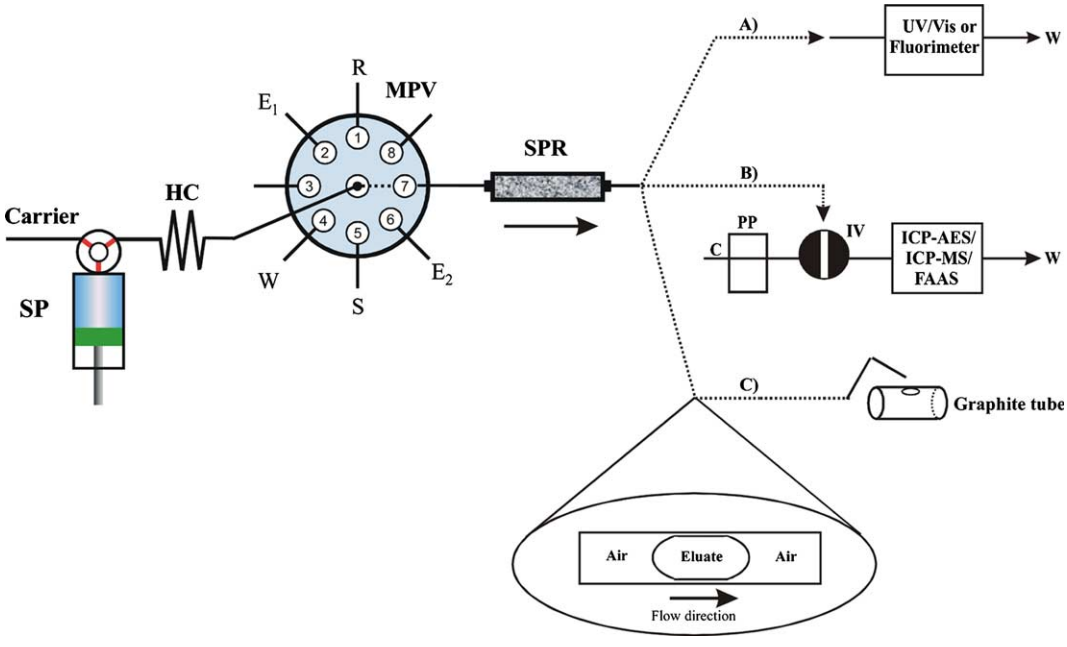

Figure 2. On-line hyphenation of sequential injection (SI) systems containing a packed bed column with various detection devices. The inset illustrates the principle of air-sandwiched elution for ETAAS detection. SP, Syringe pump; HC, Holding coil; MPV, Multiposition valve; SPR, Solid-phase reactor; S, Sample; R, Reagent; E, Eluent, PP, Peristaltic pump; C, Carrier; IV, Injection valve; W, Waste.

process. Extraction chromatographic separations tailored to an SI system should be viewed as an excellent approach to radionuclide determinations aiming to reduce drastically both analysis time and analyst intervention compared with classical separation protocols, while miniaturizing the whole analytical set-up and preventing potential health risks due to sample manipulation.

SI-extraction chromatography (SI-EC) involves incorporating a packed-bead column in the delivery line to the detection system or the fraction collector. The isolation of target radioisotopes from inactive matrix components and interfering radioactive fission products has been realized by exploiting selective chelating agents or macrocyclic ionophores physically immobilized on porous polymeric resins that are commercially available from Eichrom Industries [22]. Grate and co-workers highlighted the potential of SI-EC as a front-end to inductively coupled plasma mass spectrometry (ICP-MS) for actinide isotopic measurements [23] and to non-selective liquid-scintillation spectrometers for on-line radiometric detection, as demonstrated in the separation, identification and/or quantification of ^{90}Sr [24], ^{99}Tc [25], and actinide isotopes [26], such as Am, Cm, Pu, Th, Np and U in nuclear waste waters. Not least, the application of stopped-flow approaches for appropriate counting according to the activity level can be synchronized with the ensuing SI-sample pre-treatment protocol, and, at the same time, ensure minimal consumption of costly liquid-scintillation cocktails [24,25]. In addition, enhanced chromatographic resolution of actinide species may be accomplished by applying sequential elution approaches to the specific actinide-sorbent material, the so-called TRU resin. By proper selection of acid and complexing eluents placed at the peripheral ports of the MPV, the discrimination of actinides in different valence state groups, as well as the selective recovery of Pu and Th, via on-column redox reactions and HF/HCl elution, respectively, was shown to be feasible [26]. For ICP-MS, isobaric, polyatomic and spectral interferences encountered in the determination of actinides may be addressed by on-line hyphenation of two EC columns in series or by careful choice of chemicals for reductive sample treatment prior to analysis [23].

The versatility of SI has also been utilized for application of automated column-switching techniques [24], or sorbent-renewable approaches [27], aimed at providing fresh chromatographic columns for each sample, thus eliminating analyte carry-over problems between consecutive assays and the loss of retention capability due to irreversibly sorbed species. Although reported SI applications have mainly been focused on the analysis of highly radioactive samples, such as nuclear waste or reactor samples, SI-EC also has potential for monitoring radioisotopes in agricultural and environmental samples at a global fall-out level.

As a result of the development of porous monolithic stationary-phase substrates for separation purposes, there has been increasing interest in the possibility of conducting low-pressure liquid chromatography (LC). The bimodal porous structure of rigid monoliths, comprising small pores within a skeleton for the required interactions surrounded by larger transport channels, provides high permeability and low back-pressure, even at moderately high mobile-phase flow-rates. Particularly relevant is the fact that short monolithic columns can exhibit separation efficiencies even better than those obtained with traditional-sized particle-packed stationary phases for HPLC. Bearing these features in mind, Šatínský et al. [28] introduced the concept of SI chromatography (SIC) based on the combination of SI programmable flow for selecting the sample and the composition of the mobile phase at will with the high resolving power inherent in monolithic phases. SIC systems have commonly involved reversed-phase separations by intercalation of octadecyl-chemically-modified silica-gel monoliths (Chromolith columns) between the MPV and a flow-through diode-array or charged coupled device (CCD) spectrophotometer [28–30], which facilitates simultaneous detection at various analytical wavelengths. SIC can be operated under either isocratic conditions (by using the mobile phase as carrier) or gradient elution (capitalizing on the dispersion of the eluent into the carrier solution). In addition, automatic programming of the flow of the mobile phase by the SI instrumentation permits the application of flow gradients [29] that can enhance peak resolution while considerably decreasing the total chromatographic run-times.

Although SIC has so far been exploited for resolving organic molecules in pharmaceutical preparations, this technique has a vast potential for environmental applications, for not only organic analytes but also anion and cation separations, by exploiting ion-exchange monolith disks or reversed-phase materials physically modified with ionic surfactants, as reviewed by Paull and Nesterenko [31].

A universal SI-manifold for low-pressure chromatographic separations, as either SI-EC or SIC, is depicted in Fig. 2.

4. Solid reactors for sorptive preconcentration and/or matrix removal

SI analysis is currently viewed as a powerful analytical technique for automated sample pre-treatment and trace-level assays [32]. On-line solid-phase extraction (SPE), also called sorbent extraction, is the predominant sample-processing method that has been growing rapidly as a consequence of its straightforward operation and high separation and preconcentration capabilities. It is most often employed by using packed-bed or

disk-phase-based microcolumns, which are filled with appropriate sorptive materials and placed in the SI manifold prior to detection (position C in Fig. 1). The ultimate goal is to improve the sensitivity and the selectivity of the analytical procedure and/or to overcome the inherent low tolerance of the detection system to sample constituents by preconcentrating the analyte and/or removing interfering components from complex environmental matrices. Whenever trace analysis is pursued, matrix separation occurs concomitantly with analyte enrichment.

A multitude of solid-phase reactors with different designs, mainly uniform-bored or conical micro-columns, have been successfully implemented in SI assemblies. The temporary retention of low levels of individual metal ions, nutrients, or ionic complexes by electrostatic interactions onto ion-exchange packed-bed columns [33] or chelating reactors [34–36] (generally containing iminodiacetate moieties) has been utilized in on-line environmental assays. Derivatized non-polar chelates (mainly dithiocarbamate or dithiophosphate derivatives) or hydrophobic compounds of environmental concern have been preconcentrated on reversed-phase materials [37–40] (e.g., octadecyl-chemically-modified silica gel, polytetrafluoroethylene (PTFE) beads or co-polymeric sorbents) by partitioning, hydrophobic or $\pi-\pi$ stacking interactions. The target compounds are either directly retained on the active phase after appropriate sorbent conditioning [34–36] or derivatized into a suitable chemical form to be sorbed on the reactive surfaces [33,37–39]. The latter alternative typically gives rise to selectivity enhancement by intelligent selection of the derivatization agent as demonstrated by preconcentrating trace metals on reversed-phase materials [38,39], anion-exchangers [33] or modified copolymers [41]. However, as a consequence of the limited interdispersion between sample and reagent segments in classical SI systems, hybrid FI-SI manifolds [38] and iterative segmentation approaches [37] have been designed for appropriate analyte derivatization.

In fact, SI-SPE is playing an important role in combination with atomic spectrometric detectors, as demonstrated in several fundamental reviews [11,12,42]. The discontinuous operation of SI makes this technique well suited for hyphenation with electrothermal atomic absorption spectrometry (ET-AAS) as a consequence of the discrete, non-continuous working nature of the detector. In-line SI-SPE prior to ET-AAS can be executed in a column-in-tip mode, which is based on attaching the sorbent microcartridge at the outlet tip of the ET-AAS autosampler arm [39,43], or in a traditional column-in-manifold fashion, which uses packed-bed reactors [33–38] or open-tubular knotted PTFE tubing [44] for metallo-organic complex sorption. However, three-dimensionally disorientated (knotted) reactors have restricted application in SI due to the inherent low retention yields and moderate concentration efficiencies, the latter being a consequence of the requirement for periodical filling of the syringe pump and sample loading into the flow network. The stringent volumetric demands of the graphite platform of the ET-AAS 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 analytes ($\leqslant$50 μl) is delivered to the atomizer as a sandwich between air plugs (see Fig. 2C) [39,45].

However, the interface between the discontinuous SI-SPE systems and continuous-flow detectors, such as flame atomic absorption spectrometers (FAAS) or those offering multi-element capabilities, such as inductively coupled plasma-atomic emission spectrometers (ICP-AES) or ICP-MS, can be constructed from conventional flow-through sample injectors, such as rotary valves, as reported by several authors [35,38] and shown schematically in Fig. 2B. It should be stressed that metal-speciation analyses are compatible with SI-SPE-AAS systems by exploiting selective chemical-elution protocols, as demonstrated by Marques et al. [46] through sequential elution of Cr(III) and Cr(VI) from activated alumina-packed microcolumns.

Conventionally, the sorbent column is treated as a permanent component of the flow network, being used repeatedly for the sample-loading and elution sequences, and being replaced or repacked only after long-term operation. 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. For example, the volume changes undergone by the sorbent beads during the analytical cycle (i.e. swelling or shrinking) may progressively affect their retention capabilities. It is worse if the surface properties of the sorptive entities become irreversibly altered due to contamination, deactivation or even loss of active sites. Also, if the retained species are not totally eluted from the sorbent medium, this leads to carry-over effects between consecutive runs.

A superb alternative to overcome these drawbacks relies on a surface-renewal scheme, the so-called SI-bead injection (SI-BI) (i.e. the contents of the SPE column are withdrawn on-line and replaced for each analytical run). Originally adapted for optical sensing, SI-BI uses minute amounts of beads carrying the derivatization reagent, whereby the analyte becomes preconcentrated and monitored via optical fibers in a

jet-ring configuration or in custom-built flow-through cells (position D in Fig. 1) that capture the active sensing surfaces while allowing the perfused liquid to flow freely [47,48]. This concept of optical sensing at solid surfaces constitutes an attractive alternative to conventional procedures for eluate detection, thus preventing the partial loss of the preconcentration capabilities gained during the sorption step as a result of dilution in the eluate phase.

A further improvement in automating the microfluidic handling of suspended beads as renewable carriers of reactive moieties or immobilized reagents was recently made by introducing the μSI-LOV principle that should be regarded as an extension of micro-total analytical systems (μTASs) for on-line manipulation of real-life samples of relative complexity [17]. For trace-metal monitoring in environmental matrices by atomic spectrometry, μSI-BI-LOV systems handling either renewable hydrophilic or hydrophobic entities have been adopted as powerful sample processing ''front-ends'' to ET-AAS and ICP-MS detectors [38,49,50]. A close-up of the LOV system intended for on-line preconcentration and determination of trace metals, facilitated by means of hydrophilic cation exchangers, is illustrated in Fig 3. The current state of the art of BI in μSI-LOV prior to atomic spectrometers has been presented in review articles [18,51], wherein a vast number of approaches for manipulating the sorptive entities, on-line derivatization of target species, and monitoring of analyte-enriched beads have been thoroughly detailed and exemplified in practical applications in the environmental field.

5. Solid-sample-containing reactors

Though originally conceived for wet-chemical analysis in a miniaturized, totally enclosed environment, SI is an excellent vehicle for appropriate pre-treatment of solid samples of environmental and agricultural origin in an automated fashion. Solid samples, such as soil, sediment, fly ash, roadside dust, airborne particulate matter or sewage sludge, can be accommodated in the SI manifold as solid-phase reagents or sorbents (i.e. by filling a dedicated microcartridge incorporated between the HC and MPV (position A in Fig. 1) or attached to one of the external ports of the MPV with a representative amount of solid material (position B in Fig. 1) [52,53]).

SI is currently being exploited as a promising analytical tool for performing dynamic flow-through fractionation protocols aimed at ascertaining the bioavailability, and thus the potential toxicity, of anthropogenic metal ions in solid samples [53,54], as a complementary approach to equilibrium-based, batchwise fractionation schemes. To this end, sequential extraction protocols are readily executed on-line using traditionally-accepted extraction solutions (e.g., reagents from the three-step Standards, Measurement and Testing (SM&T) protocol endorsed by the European Commission) by exposing sequentially the solid sample contained within the microcartridge to the individual extractants attached at the various peripheral positions of the MPV [55]. Fig. 4 depicts a hyphenated SI-microcolumn system for automatic fractionation of target metal species via single or sequential extractions prior to on-line atomic spectrometric detection.

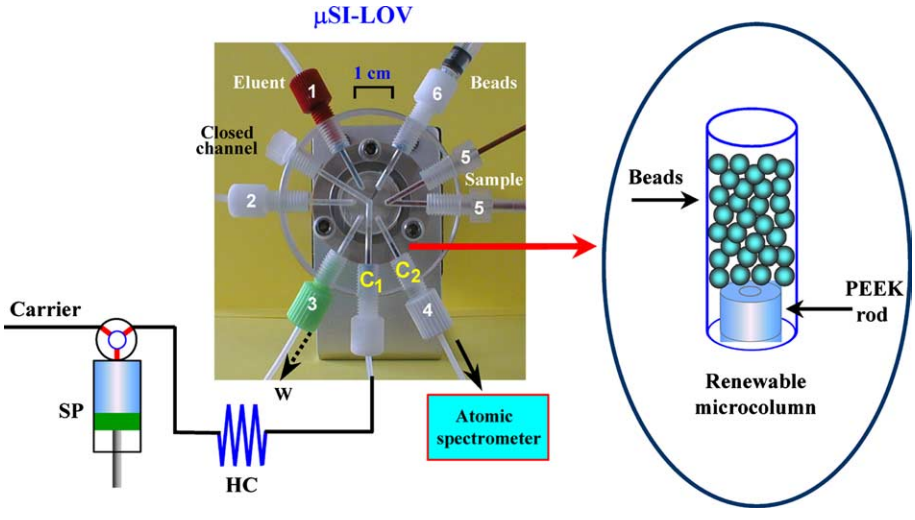

Figure 3. Close-up of a microsequential injection lab-on-valve (μSI-LOV) system for bead injection (BI) incorporating two microcolumn positions (C_1 and C_2) along with a magnified view of a packed, renewable microcolumn. In order to withhold the aspirated beads within the LOV, the column cavities are furnished with small polyetheretherketone (PEEK) roods that allow solutions to flow freely while retaining the sorbent entities effectively. SP, Syringe pump; HC, Holding coil; C_1 and C_2, microcolumn cavities; W, Waste. (Adapted from [18], courtesy Elsevier Science Publishers).

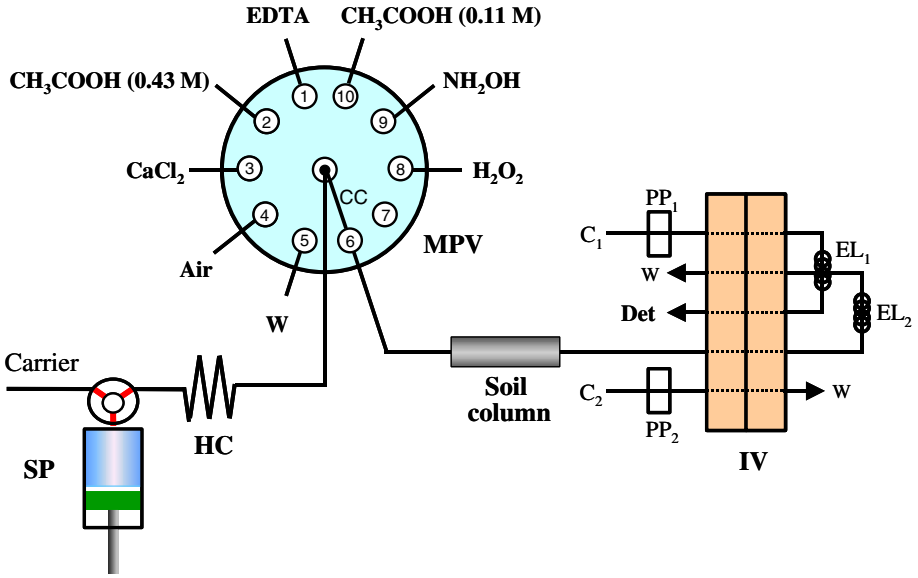

Figure 4. Schematic diagram of an sequential injection (SI)-microcolumn system hyphenated with atomic spectrometers for dynamic fractionation of metal species in environmental solids using either single extractants or the three-step Standards, Measurement and Testing (SM&T) protocol for sequential extraction endorsed by the European Commission. SP, Syringe pump; HC, Holding coil; MPV, Multiposition valve; C, Carrier; PP, Peristaltic pump; IV, Injection valve; EL, Extract loop; Det, Detector; W, Waste. (From [55], by permission of the American Chemical Society).

As compared with traditional end-over-end manual fractionation methods, this novel SI-microcolumn approach offers unrivalled features and enhanced information on the extraction steps, as summarized as follows:

(A) simplifying and accelerating the overall procedure (from days to hours);
(B) monitoring the kinetics of the ongoing leaching process for evaluating the actual mobility and accessibility of trace metals for living beings;
(C) automating the extraction procedure and detection;
(D) minimizing the risks of sample contamination;
(E) easily manipulating reagents via the MPV; and,
(F) evaluating the efficiency of the extractants and the largest pool of available fractions.

Readers are referred to a review article [56] for a thorough description of the fundamental principles of dynamic strategies for on-line fractionation analysis of trace elements in solid samples and risk assessment of environmental pollutant exposure. It also addresses the capability of SI analyzers for handling the leaching solutions in several fully-automated operational modes, according to the kinetics of metal release from targeted soil phases, and for direct and continuous analysis of extracts through synchronization with the operation of atomic spectrometers.

6. Concluding remarks and future outlook

In this review article, solid-phase reactors are presented as essential components of SI set-ups, playing a prominent role in derivatizing analytes on-line, improving mixing of sample with reagent(s), enhancing analytical sensitivity and/or selectivity, performing low-pressure chromatographic separations, and developing flow-through microcolumn fractionation studies for investigating the kinetics of pollutant leaching from environmental solids. Table 1 sets out relevant environmental applications of solid-phase-containing SI or μSI-LOV assemblies, including the role of the solid material, the type of stationary or transient phase, the detection principle, the analyte(s), the derivatization reaction, the environmental matrix, and specific features of the flow analyzers.

With respect to on-line, solid-phase-based chemical derivatization procedures for environmental assays, future trends are expected to be focused on accommodation within the SI network of catalytic heterogeneous reactions using enzyme packed-bed or open-tubular reactors. The idea behind them is to exploit the inherent selectivity of enzymatic reactions while preserving the native configuration of the protein, rather than minimizing the consumption of costly reagents, since minute, well-defined volumes of liquid-phase enzymes can be introduced into the SI microconduits via the MPV. In this context, pesticide residues (e.g., organophosphate, carbamate and organochlorine pesticides) and heavy-metal ions can be determined by the inhibitory action of immobilized enzymes, such as cholinesterases, phosphatases, and xanthine oxidases, just to mention a few.

With respect to in-line SI-SPE, enhanced tolerance to interfering compounds is obtained by either molecular

Table 1. Relevant applications of SI analyzers involving solid-phase reactors for environmental assays

Method	Solid phase	Reactor role	Analyte	Detection principle	Derivatization reaction/reagents	Matrix	Comments	Ref.
SI	Single-bead-string reactor	Zone overlapping	Boron	SP	Azomethine-H	Water effluents and fertilizer process streams	1) *In situ* preparation of the unstable chromogenic reagent 2) Exploration of various devices for promoting on-line mixing	[21]
SI	Amalgamated zinc and copperized cadmium	Derivatization (Reduction)	Fe(II)/Fe(III) and nitrate/nitrite	SP	Griess-Ilosvay and 1,10-phenanthroline complexation	Aquatic suspensions containing particulate matter and sediments	1) Use of in-line tangential filtration for determination of dissolved species 2) Suitability for field analysis and real-time monitoring	[15]
SI	Copperized cadmium column	Derivatization (Reduction)	NO_2^- and NO_3^-	SP	Griess-Ilosvay	Tap, mineral and sea water	1) Implementation of a sandwich technique for simultaneous determination of both analytes in a single sample plug 2) Reduction of nitrate in a sample sub-zone by time-controlled flow reversal	[61]
SI	PbO_2 embedded in silica gel	Derivatization (oxidation)	Mn(II)	SP	Detection of generated permanganate	Tap and domestic waters, and effluent streams	1) Careful optimization of the carrier acidity to ensure maximum solid-phase reaction efficiency 2) Design of a gas tubing for trapping the air bubbles generated during heating of carrier	[16]
μSI-LOV	Cd/Cu-foil	Derivatization (reduction)	NO_3^- (also nitrite and phosphate)	In-valve SP	Griess-Ilosvay (and Molybdenum Blue method)	Lake and tap water	1) Selection of reduction time by stopped-flow protocols 2) Bi-directional flow to minimize back-pressure effects	[19]
SI	Copperized cadmium	Derivatization (reduction)	NO_3^- and NO_2^-	SP	Griess-Ilosvay	Wastewaters	1) Simultaneous determination of both analytes by merging two lines of the MPV (one of them containing the reactor) at a confluence point 2) Improved reactor lifetime by accurate control of sample residence time and re-generation protocols using EDTA/NH_4Cl	[62]

SI	TEVA-resin (mixture of quaternary amines, i.e. Aliquat 336)	Separation	^{99}Tc(VIII)	LSc	LSc cocktail	Processed nuclear waste samples	1) Application of on-line stopped-flow radiometric detection 2) Use of a complexing eluent to remove the concomitantly-retained Pu(VI)	[25]
SI	TRU-resin (with CMPO as selective actinide complexant)	Separation	Am, Cm, Pu, Np, Th, U	LSc/fraction collection +α energy analysis	LSc cocktail for LSc detection	Nuclear waste samples	1) Elution of actinides in valence state groups 2) Selective Pu and Th elution via on-column redox with acidic nitrite and complexation reactions with HF, respectively	[26]
SI	C_{18}-silica gel	Separation	TNT	SP	Sodium sulfite/ potassium hydroxide/ acetone	Environmental waters	1) Change of reaction medium 2) Design of a field-portable process analyzer	[63]
SI	MnO_2-impregnated cotton filter	Separation	^{90}Sr and ^{226}Ra	LBPC	Direct β and α emission	Spiked drinking waters	1) Simultaneous isolation of both radioisotopes 2) Requirement for manual treatment of eluates prior to detection	[64]
SI	TRU-resin	Separation	Am, Pu, Np	ICP-MS	Direct measurements in eluate	Dissolved vitrified nuclear waste	1) Overcoming isobaric, molecular and spectral interferences 2) Optimizing reductive sample treatment to improve separation factors for U	[23]
SI	Sr-resin (with BCH as selective crown ether)	Separation	^{90}Sr	LSc/fraction collection + β-counting	LSc cocktail	Vitrified high-level nuclear waste glass and nuclear waste supernate	1) Optimizing Sr elution conditions 2) Implementing automated approaches for reliable column re-use and/or column switching for preventing sample cross-contamination	[24]
SI	Activated alumina	Separation/ speciation	Cr(III)/Cr(VI)	FAAS	Native absorbance	Effluent and natural (drinking, spring and well) waters	1) Retention of both oxidation states and selective elution 2) Requirement for large elution volumes for quantitative stripping of the analytes	[46]

(*continued on next page*)

Table 1 (*continued*)

Method	Solid phase	Reactor role	Analyte	Detection principle	Derivatization reaction/reagents	Matrix	Comments	Ref.
SI	Sr-resin, TEVA-resin and TRU-resin	Separation	^{90}Sr, ^{99}Tc, ^{241}Am	α- and β-counting	LSc cocktail	Old nuclear waste	1) Development of a single SI set-up for accommodating multiple radiometric separations 2) Exploiting the concept of renewable surfaces by coupling a set of three valves in series	[23]
SI	Anion exchanger	Separation/ preconcentration	Pb(II)	SP	Resazurine/ sulfide	Drinking waters	1) Retention of the lead(II)-iodide complex 2) Use of a stirred mixing chamber to pre-mix reagents	[33]
SI-FI	Chelex 100 (Iminodiacetate moieties)	Preconcentration/ separation	Pb(II)	SP	Malachite Green and iodide	Natural and waste waters	1) Assembling of a hybrid SI-FI system to facilitate mixing of sample and reagents 2) Implementing an anion-exchange resin to remove Cd(II) as chlorocomplexes	[59]
SI	C_{18}-silica gel	Separation/ preconcentration (column-in-tip)	Cd	ET-AAS	APDC	Drinking, tap, river and sea water	1) Use of air segmentation during metering and dispensing to prevent mutual mixing of sample, washing solution and eluent 2) Implementing a short back-suction procedure to minimize sorbent shrinkage	[39]
SI	C_{18}-silica gel	Separation/ preconcentration	Nitrite	SP	Griess-Ilosvay	Rain, tap, pond, sea and ground waters	1) Applying iterative reagent-sample segmentation for appropriate pre-column derivatization 2) Minimizing gas-evolution problems by coupling an external eluent-containing syringe pump	[37]
SI	PVP	Preconcentration/ separation	Pb	FAAS	Native absorbance	Natural waters	1) Coupling several three-way solenoid valves for loading sample, injecting eluate, and controlling the direction of carrier movement 2) Requirement for a stainless-steel filter holder to improve the stability of the polymeric sorbent	[36]

μSI-BI-LOV	Sephadex C-25	Preconcentration/ separation	Ni, Bi	ICP-MS	Direct measurement of eluate	River sediment	1) Exploiting a home-made, direct-injection, high-efficiency nebulizer 2) Implementing a pre-elution step to minimize isobaric interferences by weakly-retained metal species	[38]
SI	PTFE knotted reactor	Preconcentration/ separation	Cr(VI)	ET-AAS	APDC	Certified NIST water	1) Assembly of a two-line SI system for appropriate mixing between sample and reagents 2) Comparison of molecular sorption with on-line solvent extraction	[44]
μSI-BI-LOV	PTFE/ C_{18}-PS-DVB	Preconcentration/ separation	Cd	ET-AAS	DDPA	Natural water and river sediment	1) Investigating the feasibility of using hydrophobic materials in a renewable fashion 2) Tolerance to high salt concentrations with no need for matrix modifier	[49]
μSI-BI-LOV	Reagent modified-C_{18}-PS-DVB	Preconcentration/ separation/ speciation	Cr(VI)	ET-AAS	DPC	Sea water and hard tap waters	1) Overcoming the irreversible retention of the analyte by dissolving both the physically adsorbed reagent and complexed metal species 2) On-line pH adjustment to prevent interconversion between oxidation states	[65]
FI-SI	PTFE	Preconcentration/ separation	Cd, Pb	ET-AAS (or ICP-MS)	DDPA	Natural waters and sediments	1) Comparing the analytical performance of packed-bead columns with knotted reactors 2) Using an ancillary rotary valve as interface between the sorbent micro-column and the detector	[45]
μSI-BI-LOV	Sephadex C-25	Preconcentration/ separation	Ni	ET-AAS	Native absorbance	Industrial waste water	1) Critical comparison between elution protocols and direct transportation of loaded beads into the graphite tube 2) Low consumption of solid phase: 1 g of dry sorbent can be used for >500 analyses	[51]

(*continued on next page*)

Table 1 (*continued*)

Method	Solid phase	Reactor role	Analyte	Detection principle	Derivatization reaction/reagents	Matrix	Comments	Ref.
SI	Iminodiacetate chelators	Preconcentration/ separation	Al, As, Co, Cu, Mn, Mo, Ni, Pb, V	ICP-MS	Direct measurements in eluate	Sea water	1) Evaluating the suitability of two iminodiacetate resins with different sorbent matrix for on-line sorption protocols 2) Using an additional rotary valve for injection of eluates into the carrier stream of the detector	[35]
SI	Chelex 100 (Iminodiacetate moieties)	Preconcentration	Total dissolved iron	FAAS	Native absorbance	Tap and well waters	1) Using a hybrid FI-SI system to feed the nebulizer continuously 2) No possibility of metal speciation	[34]
SI-BI	Chelex 100 (Iminodiacetate moieties)	Preconcentration (Optosensor)	Hg(II) and Cu(II)	SPO	APDC and dithizone complexation	River waters	1) Sequential determination of Cu(II) and Hg(II) 2) Sensitivity and selectivity tuning by choice of sample volume and sorption pH, respectively	[48]
SI	XAD-8	Preconcentration (column-in-tip)	Tl	ET-AAS	Bromine	Environmental solids	1) Retention of the $TlBr_4^-$ complex 2) Multiple air-segmented elution	[43]
SI	C_{18}-silica gel	Preconcentration	BAP	VAF	Native fluorescence	Tap water	1) Using the appropriate variable-angle route to minimize spectrum overlapping with other PAHs 2) Exploiting multiple linear regression algorithms to analyze BAP in the presence of BKF	[40]

SI	Salicylic acid grafted XAD-4	Preconcentration	Al(III)	Flu	8-HQ	Potable water after Al-flocculation treatment	1) Synthesizing a selective chelating resin 2) Using a second multiposition valve for post-column derivatization	[41]
SI	SRM 2710 soil	Sample (on-line fractionation)	Cu, Zn, Mn, Fe and Ca	Fraction collection/ FAAS	Native absorbance	Highly contaminated homogeneous soil	1) Implementing the three-step SM&T fractionation protocol in a dynamic fashion 2) Evaluating four operational modes for sequential extraction: uni-directional; bi-directional; multi-bidirectional; and stopped flow	[52,54]
SI-FI	CRM483	Sample (on-line fractionation)	Cu	On-line FAAS	Native absorbance	Non-homogeneous sewage-sludge-amended soil	1) Using a bi-conical microcolumn to admit amounts of solid $\leqslant$300 mg 2) High temporal resolution of the extraction process	[55]
SI	Soil	Sample (on-line extraction)	Cr(VI)	SP	DPC	Highly contaminated soil	1) Thorough investigation of Cr(VI) to Cr(III) conversion in soil extracts 2) Optimizing sample amount and extraction flow rate to minimize bubble formation in the system	[53]

Acronyms: SI, Sequential injection; LOV, Lab-on-valve; SP, Spectrophotometry; Fl, Spectrofluorimetry; SPO, Solid-phase optosensing; VAF, Variable-angle spectrofluorimetry; FAAS, Flame atomic absorption spectrometry; ET-AAS, Electrothermal atomic absorption spectrometry; ICP-MS, Inductively coupled plasma mass spectrometry; LSc, Liquid scintillation; LBPC, Low-background proportional counter; APDC, Ammonium pyrrolidine dithiocarbamate; BAP, Benzo[a]pyrene; BkF, benzo[k]fluoranthene; TNT, 2,4,6-trinitrotoluene; MPV, Multiposition valve; BCH, 4,4'(5')-bis(tert-butylcicloheхane)-18-crown-6; CMPO, octyl-(phenyl)-N,N-diisobutylcarbamoylmethylphosphine oxide; APDC, Ammonium pyrrolidine dithiocarbamate; DDPA, Diethyldithiophosphate; DPC, 1,5-diphenylcarbazide; PVP, Poly(vinylpyrrolidone); PTFE, Poly(tetrafluoroethylene); PS-DVB, Poly(styrene-divinylbenzene); HQ, Hydroxyquinoline-5-sulfonic acid; SM&T, Standard, Measurement and Testing Programme of the Commission of the European Communities.

recognition of the target analyte through molecular imprinting or hyphenation of the low-pressure sorptive column with conventional LC systems for discriminating eluate components. The renewable sorbent (BI) concept, which has traditionally been applied to diffuse-reflectance or fluorescence-based optosensing measurements, or atomic absorption/emission spectrometric assays using jet-ring and LOV configurations, can be extended to Raman spectroscopy [57] via the rational design of dedicated flow-through cells. An alternative means for manipulating renewable reagent carriers in the SI conduits involves using magnetic microbeads that can be efficiently trapped and released from open tubular reactors by controlling the position of an electromagnet in the flow cell [58].

One of the most severe limitations of SI analyzers is the complexity of conducting pre-, or post-column derivatization reactions that can be needed to detect target species. To overcome this drawback, there have been proposals for two-line SI analyzers [44], as well as multivalve-SI assemblies [41] or hybrid FI-SI systems [25,38,59] characterized for coupling external MPVs or liquid drivers at confluence points at the outlet of the solid reactor. Another elegant solution involves hyphenating SI with the novel flowing-stream technique, called multisyringe-FIA (MSFIA) based on connecting four syringes to the same step-by-step motor, thereby allowing the simultaneous delivery of various solutions/reagents into the flow manifold, as FI systems. Further details of this technique, including the analytical background and relevant features, instrumental designs, sample-injection modes, and potential for on-line coupling to FI and SI manifolds were thoroughly addressed in an article in this journal [60].

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