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Miniaturization of environmental chemical assays in flowing systems: The Lab-on-a-Valve approach vis-à-vis Lab-on-a-Chip microfluidic devices

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Abstract

The analytical capabilities of the microminiaturised lab-on-a-valve (LOV) module integrated into a microsequential injection (μSI) fluidic system in terms of analytical chemical performance, microfluidic handling and on-line sample processing are compared to those of the micro total analysis systems (μTAS), also termed lab-on-a-chip (LOC). This paper illustrates, via selected representative examples, the potentials of the LOV scheme vis-à-vis LOC microdevices for environmental assays. By means of user-friendly programmable flow and the exploitation of the interplay between the thermodynamics and the kinetics of the chemical reactions at will, LOV allows accommodation of reactions which, at least at the present stage, are not feasible by application of microfluidic LOC systems. Thus, in LOV one may take full advantage of kinetic discriminations schemes, where even subtle differences in reactions are utilized for analytical purposes. Furthermore, it is also feasible to handle multi-step sequential reactions of divergent kinetics; to conduct multi-parametric determinations without manifold reconfiguration by utilization of the inherent open architecture of the micromachined unit for implementation of peripheral modules and automated handling of a variety of reagents; and most importantly, it offers itself as a versatile front end to a plethora of detection schemes. Not the least, LOV is regarded as an emerging downscaled tool to overcome the dilemma of LOC microsystems to admit real-life samples. This is nurtured via its intrinsic flexibility for accommodation of sample pre-treatment schemes aimed at the on-line manipulation of complex samples. Thus, LOV is playing a prominent role in the environmental field, whenever the monitoring of trace level concentration of pollutants is

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pursued, because both matrix isolation and preconcentration of target analytes is most often imperative, or in fact necessary, prior to sample presentation to the detector.

Keywords: Lab-on-a-valve, Lab-on-a-chip, microfluidics, environmental assays.

Contents

1. Introduction

While chemical assays merely a few decades ago overwhelmingly were implemented by the batch approach, precisely as they literally have been executed for centuries, the emphasis is nowadays shifted towards the use of automated, continuous-flow procedures in a miniaturized fashion. The first serious step was taken with the introduction of air-segmented flow systems [[1]] – originally especially aimed at the clinical market where the number of samples to be analysed cried for automation – followed in the mid-70's with the invention of flow injection (FI) analysis [[2]]. In contrast to earlier automated systems, which relied on physical homogenisation of sample and reagent(s) and attainment of chemical equilibrium, resulting in steady-state signals, FI was based on the measurement of transient signals. This, in turn, not

only yielded faster analysis times, but allowed the development of an array of entirely novel and unique procedures.

Taking advantage of operating under dynamic conditions, we can, as opposed to batch assays, exploit the interplay between thermodynamics and kinetics of the chemical reactions involved, which, in fact, has added an extra degree of freedom in terms of executing chemical assays. Just to mention a few, we can point to [3-5]: (i) the practical exploitation of bio- and chemiluminescence detection, the coupling of which to FI has been termed to constitute the "ideal marriage" because of the reproducible and accurate timing of sample processing in the microconduits of the flow network; (ii) the viable monitoring of short-lived, meta-stable constituents in lieu of the ultimately formed reaction products; (iii) the application of kinetic discrimination schemes as utilised, for instance, in hydride-generation protocols for metalloid species to minimize the interfering effects in the conventional batchwise procedures arising from the concomitant presence of transition metal ions; and (iv) the performance of sophisticated enzymatic assays aimed either on determining selected substrates or measuring enzyme activities, which by conventional means are rather cumbersome to facilitate, yet in FI are relatively simple to accomplish.

As a consequence of the growing environmental demands for reduced consumption of sample and reagent solutions and for the development of rugged analyzers aimed at environmental monitoring purposes with capabilities for multi-analyte determinations, the first generation of FI was in 1990 supplemented by the second generation, that is, sequential injection (SI) analysis [6,7] based on discontinuous programmed flow. And in 2000 it was extended by the third generation named the Lab-on-a-Valve (LOV) [8]**,** which was initially spawned as a fluidic universal system for downscaling reagent-based assays to the micro- and submicroliter level. Yet it has concurrently proven to offer vast potentials for accommodation of a wide variety of sample processing steps in a micro-scale according to the requirements of the assays.

The second generation of FI capitalizes on the exploitation of a multi-position selection valve, the central port of which via a holding coil is connected to a syringe pump operating as the liquid driver. Thus, through the central communication line and the valve's internal rotary conduit the syringe pump can be made to address each of the ports of the valve, from where precisely metered zones of individual fluids can be aspirated into the holding coil, in which they are stacked as plugs one after the other. Afterwards, the segments are propelled forward towards the detector, undergoing on their way dispersion and thereby partial mixing with each other, and hence promoting chemical reaction, the resulting composite zone being monitored

downstream by a flow-through detector. Fully computer-controlled, the SI assembly implies substantial savings in regard to forward-flow FI set-ups, not only in consumables, but also inherently in waste generation which has become of special concern considering the increasing costs for waste disposal.

It is characteristic that the developments of the three generations of FI, resulting in miniaturization of the manifolds, were made by chemists, due to evolving demands. Either as dictated by practical considerations, such as reduction in consumption of expensive and/or rare reagents and limited sample volumes, or as required by the particular chemistries to be executed. Within the past decade, a number of research institutions, predominantly (but not exclusively) manned by electrical and mechanical engineers, have parallelly and intensively focused on miniaturisation of flow systems, which has resulted in the development of the so-called micro total analysis systems (μTAS) [9], or as they lately have been termed Lab-on-a-Chip. An example of such a microfluidic system is shown in Fig. 1. The channel network, which is made by various sophisticated procedures, such as micro-drilling, etching, photolithography, or laser erasing, is impressively exact and reproducible, allowing different channels profiles to be obtained. In many instances it can be made in inexpensive materials, namely silicon, glass, polymethyl methacrylate and polydimethylsiloxane, and mass-produced at low cost, in fact, at much lower expenditures than the LOV. However, the microfluidic devices are usually dedicated, that is, they have fixed architecture for predetermined chemistries. Readers are referred to the following comprehensive reviews [10,11] and monographs [12-15] for a thorough description of microfabrication technologies for microfluidic systems, chip components (namely, microvalves, micropumps, and interfaces to detectors), along with analytical standard operations including injection, fluid handling, reactors and mixers, separation, and detection, and relevant (bio)analytical applications as well.

In recent years there has been much attention on developing these systems for practical chemical assays in various bioscience/analytical science fields, such as DNA-separation, analysis and sequencing [16,17], clinical diagnosis [11], immunoassays [16], proteomic and cellomic analysis [14,18-20] and environmental monitoring [21] as well. Yet, to the best of the authors' knowledge the analytical features of the miniaturised chips have not been critically compared so far with those of the LOV assemblies. This review article is thus aimed at discussing the pros and cons of the LOV scheme vis-à-vis LOC microdevices, with particular reference to environmental chemical assays.

2. Peculiarities of the lab-on-valve microfluidic system

The LOV approach should be viewed as a judicious advance towards the automation of microfluidic handling of samples, alike in μSI networks, but within integrated microbore units. The microconduit unit, made initially of Perspex, but more recently of hard polyvinylchloride, polyetheretherketone (PEEK) or polyetherimide (ULTEM) for improved chemical resistance to a wide range of organic solvents, is a single monolithic structure mounted atop of a six-port selection valve, as illustrated in Fig. 2. Designed to incorporate all necessary laboratory facilities for a variety of analytical chemical assays, hence the name *lab-on-a-valve*, it is made to contain mixing points for sample and reagents; working channels for sample dilution, overlapping of zones and sample purification; and a multipurpose flow-through cell for real-time monitoring of the development of the chemical reactions [22]. In fact, the LOV unit is devised to incorporate detection facilities, that is, optical devices (namely, diode-array spectrophotometers, charged-coupled devices (CCDs), laser-induced spectrofluorimeters or luminometers) where the communication to the detector and/or the light source are made via optical fibres (see Fig. 3), and where the position of the fibres can be used to adjust the optical light path of the cell [8]. The microfabricated channel system is also amenable to admit conventional sized peripheral devices, thus facilitating the hyphenation with a plethora of modern detection techniques/analytical instruments, such as electrothermal atomic absorption spectrometry [23,24], cold-vapor atomic spectrometry [25], electrospray ionization mass spectrometry [26,27], atomic fluorescence spectrometry [28,29], inductively coupled plasma-mass spectrometry [30], and most importantly, to column separation systems, such as capillary electrophoresis or high-performance liquid chromatography, for multiparametric assays [31-33].

A valuable asset of the microflow structure is the microfluidic handling of not only metered volumes of solutions but solid suspensions as well for exploitation of heterogeneous chemical reactions. The LOV approach fosters the in-valve manipulation of sorbent materials carrying suitable surface moieties in order to generate packed column reactors for micro-scale solid-phase extraction [34,35], including ion-exchange, chelation or hydrophobic interactions, in a permanent or a renewable flow fashion, that is, the so-called bead-injection scheme [36,37], depending on the particular chemical assay. In short, microcolumns are in-situ generated by aspirating beads with particular surface characteristics and particle sizes, advantage being taken of the fact that the sorbent can be manipulated exactly as when handling liquids. The solid entities can even be automatically transported between different column positions within the LOV, their retention within the columns, as shown in Fig. 2, being facilitated by fitting the

column positions with appropriate stoppers, which will keep hold of the beads, yet allow solutions to flow freely. Following sample loading and clean-up protocols, appropriate eluents can be aspirated, and the eluate propelled to either the flow-through cell or an external detection device, as sandwiched by air or immiscible liquid segments in order to preserve its integrity. The multipurpose flow cell can even be configured to admit bead particles, thereby serving as a platform for real-time monitoring of chemical events at solid surfaces [8], the exploration of cellular activities via immobilized living cells [38,39] as well as the investigation of biomolecular association and dissociation processes [26], as exploited in enzyme-linked immunosorbent assays [40] and affinity chromatographic methods [41]. Since the entire protocol sequence is computer controlled, all fluidic unit operations are readily to be re-programmed according to the involved chemistry.

As a result, the micromachined unit is currently being advantageously used as a ”front end” to execute appropriate sample pretreatments as demanded in environmental assays, such as matrix isolation, analyte preconcentration and derivatization reactions aimed at introducing the analyte optimally into the internal/external detection apparatus [22].

3. Analytical capabilities of μTAS vis-à-vis LOV

While the dimensions of the channels in the μTAS microfluidic systems are wide-ranging but merely covering the span from depths of the order of 10-100 μm, the corresponding channel dimensions in the microfabricated LOV unit are typically ranging from 0.5 to 2.0 mm. When comparing these two devices, one may then ask, what is the crucial difference between the two systems? Intuitively, the response would be to point to the channel dimensions, as a consequence of the large size discrepancy, which amounts to 1-2 orders of magnitude. The downscaling of flow path in μTAS has undoubtedly revolutionized the volume requirements of (bio)chemical assays, leading to chips able to processing of samples within the nL to pL range, thus facilitating the implementation of single-molecule detection methods [42].

According to the literature, trends within the bioanalytical field are directed to the on-line separation, selection and digestion of target proteins for further identification by mass spectrometry [20]**.** The handling of macromolecules in samples containing suspended cells or colloids is however getting increasingly troublesome as the microfabricated channels become narrower and longer as a result of channel clogging, surface contamination, sorption of target

species (e.g., proteins) or creation of unpredictable surface potentials. On the other hand, turbid and particle containing samples, and even bead suspensions with particle sizes ~ 50-100 μm, would not pose a problem in LOV microfluidic systems as a consequence of the relatively large bore conduits in the monolithic structure.

In the author's opinion, however, the crucial difference between both microfluidic systems is rather associated to the means for fluid motion within the microchannels, that is, the propelling device. Liquid manipulation in μTAS systems to a large extent has been based on electroosmotic or electrophoretic forces [43,44], which, in turn, set certain requirements on the chemical composition of the solutions handled, but also on surface tension or free transverse diffusion [15]. Although pressure driven flow is also applicable by implementation of micromechanical pumps within the microdevices, electroosmotic pumping has the inherent advantages to be pulse free, with no backpressure effects as occurring with integrated pumps and offers an extra degree of freedom as regards to improved miniaturization [21].

On the other hand, fluid movement in LOV capitalizes on mechanically driven flow as precisely executed via an external microsyringe pump. Recent microchip devices are also amenable to piston pumps [45], yet the unrivalled feature of microfluidic handling via the μSI-LOV mode, as opposed to the conventional continuous-flow operating μTAS, is the application of flow programming based on bi-directional flow, but also, and not the least, on stopping-flow approaches, for all unit operations for any length of time completely at will.

Thus, we are not being dictated by the custom-built system in order to implement our chemistries, but we are in LOV controlling the parameters in order to adapt the physical movements of the liquids to the chemistries to be implemented. This, very importantly, implies that we can intelligently exploit the interplay between thermodynamics and kinetics. Said in other words, while we are in control of the fluidics, we can adapt them to the chemistry taking place, which, in turn, essentially gives us an extra degree of freedom. And this is of utmost relevance in executing different assays, especially if we are dealing with chemistries that are not fast or instantaneous, or even require stepwise reaction sequences. In this context it is interesting to note that the authors of μTAS articles are customarily demonstrating the capacity of their LOCs for fast, single step chemistries (re the old batch assays), mostly in the biosciences area [15], which leaves a multitude of very interesting and intriguing chemistries unexplored. Most importantly, μSI systems and novel flowing stream approaches such as multicommutation [46,47] and multi-syringe [48,49] and multi-pumping [50] flow analysis have opened new avenues as regard to controlling and enhancing the mixing

degree of sample and reagents at the microscale level. Actually, recent efforts in the μTAS field do focus on the resorting to binary sampling and tandem flow in multicommuted flow systems as a versatile means for stacking well defined plugs of sample and reagent(s) in the microchannel network [51].

Microfluidic devices have also found their place within the environmental field, as recently pinpointed by Marle and Greenway in a fundamental review [21]. This is a consequence of their in-field real-time monitoring capabilities valued from their miniaturized size, ready portability and use for remote operation. The analytical results are therefore available earlier at high temporal and spatial resolution, and at low cost, with no need for further transportation of the samples to the chemical laboratory. The development of integrated microsystems for environmental monitoring has however launched the so-called "world-to-chip" dilemma, which casts doubts upon the real applicability of microchips for real-life samples [52]. There is often, on one hand, no limitation as regards to the available volume of environmental sample as opposed to assays in the forensic, clinical and bioanalytical areas. On the other hand, the complexity of the environmental matrix and the low level concentrations of target pollutants to be continuously monitored, as endorsed by existing directives, call for sample processing steps prior to presentation of the species to the detector system that are currently regarded as the Achilles' Heel of the μTAS concept for direct analyses of real world samples. Not to forget that the downscaling of processed sample volumes to the low nL level might question the reliability of LOC results as a consequence of the lack of representativeness of the small sample in regard to the bulk medium.

In the following we will describe, via selected examples, the potential of the LOV scheme to tackle the abovementioned drawbacks of microflow structures while demonstrating their open-architecture via programmable flow to accommodate unique environmental analytical applications. Such applications are, in our opinion, not feasible to do, at least at the present stage, in μTAS, as a consequence of their inability to exploit kinetic measurements and discrimination schemes, and to handle complex sample pre-treatments.

4. Relevant features of μ-LOV devices

4.1. Versatile analytical standard operations

The analytical procedure for any chemical assay involves a sequence of operations that start with sample metering and progress, in the simplest case, to reagent additions, mixing, and a

final detection step. Additional mandatory processes might comprise appropriate sample conditioning or multiple-step derivatization reactions. The ultimate aim of any microfluidic system is the automated performance of these operations reliably, countless times, in a reproducible fashion, while sample cross contamination should be kept negligible.
In continuous-flow based manifolds, such as FI, the accommodation of different analytical protocols is accomplished via the physical arrangement of individual components, such as valves, mixing points, reaction coils. Yet, changing of any flow component in dedicated, microfabricated chips entails the complete redesign of the microchannel manifold.

The lab-on-valve manifold, however, uses a universal hardware configuration for all analyses, thus merely requiring changes in the software protocol, and the components (e.g., reagents, external modules/detectors) can be accessed randomly via appropriate computer control. Actually, the individual unit operations are in LOV clustered around the selection valve [53], and the sample zone is transported from one unit operation to the next one to implement the desired analytical methodology as described below.

4.1.1- Sample injection

To introduce minute but reproducible sample volumes into a microfluidic device, diverse strategies encompassing the use of time-based or discrete volume-based electrokinetic injection, pressure injection or mechanical injection via microrotary valves are worth to mention [54].

The LOV microfluidic unit, however, offers a universal means for sample introduction as a result of its singular hardware configuration [8]. The central processing unit in the LOV monolithic structure has been designed to house a flow-through port (see port 5 in Figures 2 and 3), where one channel serves as the sample solution inlet while the other channel works as the sample outlet, which is plugged to an ancillary peristaltic pump. Sample injection is effected by directing the central communication channel of the multiposition valve to the flow-through port followed by precise reverse motion of the syringe pump. The peristaltic pump permits the sample conduit to be thoroughly washed between standards and samples of different concentrations concurrently with the execution of the analytical procedure, thus preventing carryover effects whilst assisting in increasing the sample throughput as compared with μTAS. In the latter, the time needed for chip conditioning after the (bio)chemical assay should be taken into consideration as being frequently the limiting step of the overall analytical procedure. Sample consumption in LOV is greatly reduced via time-controlled activation of the peristaltic pump.

When dealing with multi-analyte determinations via on-chip capillary electrophoresis separation and further on-line detection, the programmable μSI protocols are able to control the entire suite of the system's peripherals, namely syringe pump, power supply and isolation valve [32], to conduct automatically, upon desire, various types of sample injections including electrokinetic injection, hydrodynamic injection and head column field amplification sample stacking injection [31].

Though originally conceived for liquid-phase assays, the direct introduction and treatment of solid samples of environmental and agricultural origin plus processing of resulting extracts might be also accomplished in an automated fashion via LOV microfluidic operations as recently demonstrated in the development of flow-through, dynamic fractionation schemes for solid substrates as contained in dedicated microcolumns embodied to the microflow assembly [55,56]. In contrast, there are limited applications for microfluidic LOC devices in analysing soil matrices owing to the inherent complexity of sample introduction and the requirement of pre-treatment protocols prior to on-chip analyte detection.

4.1.2- Sample processing

The most severe limitation of microfluidic devices for environmental surveillance is the hindrance in handling complex matrices as a consequence of channel clogging when introducing suspended particles. The immediate consequence is that LOC systems cannot readily admit micro-scale solid-phase extraction (μSPE) protocols for on-line processing of complex matrices containing trace level concentrations of target compounds.

The LOV concept has emerged as a convenient front end to facilitate automated μSPE procedures, which yield high concentration factors and minimum consumption of organic solvents [24,57]. Alternatively, both precipitates and co-precipitates generated on-line might be conveniently handled within the LOV microchannels and preconcentrated by chemical and/or physical immobilization onto sorbent reactors [28,58]. In conventional FI column preconcentration systems the sorbent-packed column is employed as an integral component of the flow network which hinders reliable long-term unattended operations as a result of the progressive tighter packing of the sorbent bed, cross contamination effects and the malfunction of the reactive surfaces due to the leakage of sorbent moieties and/or irreversible sorption of matrix ingredients. The aforementioned drawbacks can be alleviated by adapting the concept of renewable surfaces described above where the on-line packed microcolumns are renewed after each analytical cycle. Readers are referred to the following critical review

papers for an in-depth description of the potential of LOV bead-injection microsystems for monitoring of trace metal concentrations in environmentally relevant matrices [22,34,[59]]. Current emerging trends in the field are devoted to the replacement of non-selective ion-exchangers or chelators by hydrophobic surfaces, because, via the intelligent choice of the ligand used for generation of non-charged organometallic compounds, it is possible to design dedicated, selective chemistries for trace elements with negligible interfering effects arising from major matrix elements, namely alkaline and alkaline-earth metal ions. At this juncture, a recent work dealing with the determination of trace level concentrations of Cr(VI) utilizing poly(styrene-divinylbenzene) beads containing pendant octadecyl moities (C_{18}-PS/DVB) as microcarriers for the chromogenic derivatization reagent proves that there is a crucial need to get knowledge on the yield and the kinetics of the heterogeneous derivatization reactions for appropriate performance of the microanalytical flow systems [[60]]. Indeed, the LOV set-up configured in a bead-injection spectroscopic fashion might be regarded as an excellent tool for the examination and optimization of immobilization protocols for target ligands on bead surfaces [[61]].

The scope of the LOV scheme for environmental monitoring of pollutants at trace levels have most recently been expanded from inorganic analytes to persistent/pseudopersistent organic compounds, such as pharmaceutical residues in waterways. The microanalytical system has proven itself as a straightforward and cost-effective alternative to currently available robotic sample processors (e.g., Prospekt-2 and Symbiosis from Spark Holland or OSP-2 from Merck) comprising exchangeable cartridge modules for single use SPE columns [[62],[63]] prior to liquid chromatographic separations, as demonstrated by the accurate determination of non-steroidal anti-inflammatory drugs and lipid regulators in wastewaters with no need for preliminary batch sample pre-treatments [33].

Miniaturization of assays based on generation of hydrides or volatile species linked to the advent of miniaturized spectrometers, such as plasma on a chip, has led to the integration of gas-liquid separators; e.g., the Venturi and gas-expansion separators, within the LOV module for conferring a portable analyzer encompassing on-line sample processing [25]. In addition to the benefits of chemical vapour generation - embracing the separation of analytes from complex matrices, analyte enrichment, and fast reaction speed - and those of miniaturization via μSI-LOV programmable flow, rendering decreased sample and reducing reagent consumption, interfering effects from transition metals ions can to a large extent be reduced by judicious exploitation of kinetic discrimination schemes, that is, even subtle differences in the reaction rates of occurring chemical reactions may be used for analytical purposes [5].

Because of the precisely-controlled hydrodynamic conditions in the flow network and short residence time of the sample plug within the system, possible side reactions can be kinetically discriminated at the expense of the main reaction for evolving gaseous species [29].

Liquid-liquid microextraction procedures, commonly referred as to single-drop solvent extraction [64] or hollow-fiber supported extraction [65], are to be gaining full automation when translated into a μSI-batch fashion. The microfluidic system automatically performs the steps of derivatization of the analyte, if necessary, exposing the chemical modified sample to a suitable extractant, mixing of sample and reagents into an extraction coil, separating the immiscible zones and transportation of the extractant zone to a detector for analytical measurements. The programmed forward-backward movement of well-defined stacked zones in the extraction reactor ensures rapid and efficient phase transfer which is assisted by the thin-film tube-wetting characteristics of the extractant. The lack of reliable determinations in FI forward-flow systems, commonly attributed to the inefficiency of dynamic separation and recovering of the phase of interest free of the immiscible liquid, is alleviated in μSI extraction systems [66]. Actually, the discontinuous flow pattern inherent to the SI concept readily facilitates the separation of immiscible phases under steady-state in lieu of dynamic conditions by delivering of the stack of zones to a conical separation chamber clustered at the multiposition valve as a peripheral manifold component [53,67].

4.1.3- Fluid handling

The key to downscaling in LOV is the replacement of continuous flow from LOC microdevices by programmable flow, which will move both liquids and gases when and where they are needed in a user-friendly fashion, by stopping, reversing and accelerating flow rates. Though it would ostensibly seem that the permanent rigid position of the flow path and confluences in the LOV monolith, alike dedicated microchips, detract from flexible microfluidic manipulations, the microbore unit is amenable to execute any desired unit operation at will by selecting the amplitude of the flow reversal in the holding coil, and most importantly, by random access to the desired peripheral modules or detection devices. Thus, for example, controllable dispersion of the sample zone, leading to a wider dynamic linear range, is readily achieved by programming forward-backward flow protocols of the stacked sample and carrier plugs in the holding coil [68], or by delivering a precisely metered sample zone to an external mixing chamber when seeking for higher dilution factors. In other instances, however, separating zones with immiscible fluids are adopted for transportation of the sample plug from one unit operation to the next to preclude undesirable dilution. Such

bracketed zones experience mixing patterns typically found in segmented flow analyzers, that allow measurements under steady-state conditions [69]. Though the penetration of air into the fluidic channels in μTAS systems is undesirable for convenient pumping or delivery of solutions within the chip [70], air bubbles are often introduced into the microbore LOV structure for creating a miniature well-mixed environment, constituting the basis of the coined monosegmented-flow analysis approach [71,72]. Air segments are also most appropriate for delivering of a discrete volume of fluid, as demanded, for example, in micro-scale SPE hyphenated to electrothermal atomic absorption spectrometry to meet the restricted volumetric requirements of the graphite platform of the atomizer (<50 μl) and the reliable accommodation of the eluate within the tube [58,60].

Whilst LOCs are tailor-made for a specific task, the most salient feature of LOV is their universal applicability for a breadth of wet chemical assays involving either homogeneous or heterogeneous chemical reactions. This is of particular importance in process analysis in the biotechnological field, where multiple analyses are needed in almost real time with minimum human intervention and using minute sample volumes [73,74].Yet also in environmental monitoring, where high-resolution temporal and spatial data for a suite of chemical parameters (e.g., nutrients, major and trace elements) need to be obtained to acquire knowledge of the processes occurring under natural conditions [75]. As opposed to their continuous-flow counterparts, reactions with divergent kinetic demands can be easily implemented in a single LOV protocol sequence. This has been neatly exploited by Wu and Ruzicka [76] for accommodating and optimizing EPA-approved methods for in-valve spectrophotometric determinations of nitrate, nitrite and orthophosphate without manifold reconfiguration. A copperised cadmium-foil filled miniaturized microcolumn is incorporated for on-line reduction of nitrate to nitrite prior to further reaction with the Griess-Ilosvay reagent. Precise fluidic control is here needed for ensuring an acceptable yield of the heterogeneous reaction while preventing further overreduction of the target analyte to ammonium. Full benefit from programmable flow is also to be obtained when handling unstable reagents in solution as generated in-line at solid-phase redox reactors [77]. To increase the sensitivity for kinetically slow reactions, the overlapped reagent/sample zones can be monitored by adopting the stopped-flow approach, the effectiveness of which has been illustrated in the LOV determination of orthophosphate at the low ng/ml level in surface waters [76].

Regarding the separation and preconcentration of trace levels of metal ions by adsorption on reversed-phase sorbent materials following on-line dynamic derivatization, it was found

that in many instances a certain delay time had to be implemented, giving the reaction sufficient time to generate the complex, which then, in turn, could be adsorbed on the solid-phase bead material [78]. For the very same reason, an LOV-manifold such as the one shown in Fig. 4 was used, where an external reaction coil (RC) is attached to one of the peripheral ports of the valve. Briefly, the aspirated sample is initially merged with a chelating reagent and guided to RC where the generation of a non-charged complex takes place, whereupon the reaction product, following backward aspiration, is exposed to the bead material. Subsequently, the metal chelate is eluted with a well-defined plug of a water-miscible alcohol, and then transferred to the atomic spectrometer for quantification. In this unit operation, it is frequently observed that the dynamic elution seldom renders quantitative stripping of the retained analyte, but this is readily amended by incorporating a user-defined stopped-flow period of the eluent within the renewable packed column reactor [33,78]. Thus, by appropriate programming of the method operandi, it is feasible to adapt the miniaturised flow system to the requirements of the chemistry with no further hindrance.

The use of an external RC for conducting a necessary chemical operation was also recently reported for the speciation analysis of Cr(III) and Cr(VI) at trace levels using a single hydrophilic microcolumn, namely, a polysaccharide material with covalently immobilised iminodiacetate moieties, that is, it can complex and retain Cr(III) ions. The procedural approach involved the direct determination of Cr(III), and the sum of Cr(III) and Cr(VI) being afterward quantified via on-line reduction of Cr(VI) to Cr(III) [79]. The on-line reduction was effected by on-line merging of the sample zone with hydroxylamine, yet although this was the optimal reagent of a series of reductants assessed, e.g., ascorbic acid and hydrogen sulfite, it reacted rather slowly, requiring around 4 min for accomplishment of an acceptable reduction yield. Yet, as the detector used was ETAAS, this delay time did not impair the sample throughput, because while the Cr(VI) contained in an aspirated aliquot of sample was reduced to Cr(III) as effected in the external RC via the stopped-flow approach, the indigenous Cr(III) could, after preconcentration on the beads and separation from the matrix constituents and subsequent elution, be determined through the ca. 4 min long temperature program of the graphite atomizer. When the measurement was completed, the reduced sample was ready to be subjected to the same treatment, and the total Cr-content quantified. Again, by playing on the proper timing, all reactions could be individually optimized, and the analytical protocol cycle greatly accelerated, regardless of the type of reagent-based assay [80]. Alternatively, and taking into account the different nature of both oxidation states, selective sorptive

preconcentration of both Cr(III) and Cr(VI) might be accomplished by packing two of the micromachined channels with chelating and anion-exchange beads, respectively.

In contrast to microchip devices that are typically furnished with integrated pumps and valves, the LOV unit is amenable to any desired flowing stream approach for fluid handling. Though it has been extensively linked to μSI, it should be born in mind that this marriage, whenever utilized for bead-injection analysis, lacks flexibility for on-line manipulation of the eluate following μSPE within the valve microconduits. As to on-column extraction schemes for hydride-forming species, post-column analyte chemical derivatization for evolving of gaseous species is a must [81]. And the on-line hyphenation of reversed-phase SPE with HPLC for monitoring of trace level concentration of organic pollutants needs dilution of the alcoholic eluate with aqueous solutions to prevent the broadening of the injection band along the analytical column [82]. In this context, the multisyringe flow injection (MSFI) analysis approach [48, 49], combining the advantages of multichannel operation for convenient processing of the eluate, pulseless flow, and the accurate metering of microvolumes of solutions via multicommutation protocols, has proven an appealing alternative to μSI for accommodation of LOV methods requiring the processing of the eluate prior to detection [29, 33]. Hybrid μFI-SI analyzers composed of two or more individually-operating syringe pumps are also reported to constitute a versatile means to house LOV procedures [24,28]. The simultaneous rather than sequential time-based propelling of sample and reagent segments improves zone overlapping as compared to conventional μSI systems relying on axial controlled dispersion [79,83].

Based on merging the propelling channels of the various liquid drivers at affixed confluence points, the hybrid microflow systems are superb for on-line sample conditioning (e.g., pH adjustment) prior to further sample processing in the LOV module [24]. Not only for satisfying maximum reaction yields, but also for preventing time-dependent interconversion between oxidation states of target species, that might have occurred whenever performing the assay in a batch fashion [84]. For example, the reduction of Cr(VI) to Cr(III) in natural waters by dissolved organic matter is known to be catalyzed by the presence of oxonium ions, that, however, are required for analyte derivatization whenever exploiting the 1,5-diphenylcarbazide (DPC) chemistry [60]. Yet, the reaction in acidic media is rather slow, and therefore it can be neglected by on-line acidification of the sample immediately prior to its exposure to the DPC reagent.

4.1.4- Detection

The two main on-chip detection schemes employed in microfluidic systems for environmental monitoring are electrochemistry and spectrophotometry. Custom-built electrodes are straightforwardly implemented into microchip systems to provide simple, low powdered, cost effective detection methods exploiting amperometry, voltammetry, coulometry or conductometry [21]. Particularly remarkable is the contactless conductometric detection utilising external electrodes that simplifies the construction of the microchip whilst preventing electrode fouling. Electrochemical detection has been mostly coupled to on-chip capillary electrophoresis separations [10,11]. The separation channel is fully integrated within the microfluidic device, the rigid architecture of the chip being merely suitable for dedicated, user-defined applications. In contrast, the capillary and detector in the LOV system are not integral parts of the microflow structure, but peripheral components of the manifold [31,32]. The μSI fluidic handling system might even be programmed for the preparation, conditioning and reactivation of the capillary, fast electrolyte exchange and automated sample injection by electric field and /or by pressure, thus again denoting the versatility of the monolithic module for implementing unit operations upon demand. The LOV module might also be designed to work as a flow-through potentiometric or voltammetric cell by inserting all-solid-state electrodes into the valve ports for both dynamic and static measurements [85], or alternatively admit peripheral purpose-made electrochemical cells housing the electrodes, thereby rendering the so-called Lab-at-valve approach [86].

Although the development of a wide range of intense light-emitting diodes that can be coupled to fiber optics has enabled the integration of spectrophotometers within microfluidic devices, on-chip spectrophotometric detection lacks sensitivity for trace level analysis as a consequence of the processing of sample volumes at the low nL or pL level and miniscule channel dimensions which render optical path lengths < 1mm [21,51]. On the other hand, the multipurpose LOV flow-through cell furnished with optical fibers admits larger sample volumes, the application of on-column sorptive preconcentration/detection (bead-injection spectroscopy) protocols and is to be readily configured for absorbance, fluorescence or reflectance measurements by manual positioning of the outlet fiber [8,87,88]. A singular asset of the flow-through cell is that the optical path length is not affixed to a particular value rather it can be extended, according to the needs of the assays, up to 10 mm by tailoring the liquid gap between both optical fibers. Chemiluminescence detection has also been described by employing newly designed LOV microsystems hyphenated to Z-type flow cells [77]. Notwithstanding the discontinuous-flow nature of μSI-LOV analysis, there is no limitation for

hyphenation to either continuously or discontinuously operating external atomic absorption spectrometers, including flame atomic absorption spectrometry, inductively coupled plasma-atomic emission spectrometry, inductively coupled plasma-mass spectrometry, atomic-fluorescence spectrometry, and graphite furnace atomic absorption spectrometry as well, via appropriate interfaces, for detection of trace metals and metalloids following in-valve pre-treatment and/or on-line derivatization reactions [22,34]. Figures of merit of relevant LOV-based microfluidic methods for environmental applications, including detection system, analyte(s) type, environmental matrix, dynamic linear range, detection limit, precision and the potential utilisation of on-line sample processing protocols are compiled in Table 1.

5. Concluding remarks

In this article, the microfluidic handling capabilities of LOV systems aimed at the implementation of unit operations have been critically compared with those of LOC microdevices for environmental assays. It is well recognized that the microchip technology is to date unable to satisfy the current demands as regards to micropollutant monitoring. The most severe limitations arise from the introduction of environmental matrices, the small sample volumes processed, the forward-flow pumping of solutions within the microchannels, the matrix interferences and the high limits of detection obtained. And, not the least, the lack of being able to exploit the interplay between thermodynamics and kinetics of the chemical reactions taking place.

Despite the attempts for conducting on-chip sample pre-treatments, it is evident that there is a need for an efficient interface between real-life samples and the microfluidic device. Actually, the world-to-chip dilemma might be readily resolved as discussed in the bulk text by utilizing the LOV microsystem as a front-end to microchips. In flow-through LOV analyzers there is no restriction as to the handling of aqueous solutions, particle-containing matrices or solid samples via in-valve sample processing operations and/or the implementation of external modules. Larger sample volumes/amounts may be processed in a bi-directional flow fashion that facilitates the mixing with reagent zones for chemical derivatization reactions, while ensuring sample representativeness. And micro-scale SPE with renewable surfaces has been extensively used (see Table 1) for isolation of target analytes from matrix ingredients with concomitant sorptive preconcentration onto the bead material.

Current research in the environmental field is being focused on the hyphenation of LOV microdevices in an SI, MSFI or multicommutation-flow format to column separation systems,

such as capillary electrophoresis, liquid chromatography and gas chromatography, coupled to mass spectrometers for development of fully automated multiresidue methods. The injected sample can be processed or reacted in the LOV device prior to electrophoretic or chromatographic separations for appropriate matrix clean-up/on-line analyte enrichment, and different post-separation reagent-based assays might be also accommodated in the same set-up prior to detection via computer-controlled fluidic manipulations.

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Figure captions

Figure 1- Magnified close-up of a microchip structure designed for electroosmotic flow pumping. (Reproduced from the Homepage of the Department of Micro and Nanotechnology, Technical University of Denmark (http://www.mic.dtu.dk/English/Research/BCMS.aspx)).

Figure 2- Illustration of a μSI-LOV microflow network as assembled for in-valve sorptive preconcentration using renewable sorbent materials prior to further detection via peripheral analytical instruments. SP: Syringe pump, HC: Holding coil. The insert at the right shows how the sorptive beads are retained within the column positions (from Ref. [22], courtesy Elsevier Science Publishers).

Figure 3-Schematic diagram of a μSI-LOV manifold furnished with a multi-purpose flow cell as configured for optical measurements including bead-injection spectroscopy. SP: Syringe pump, PP: Peristaltic pump (from M. Miró and W. Frenzel, Flow Injection Analysis: Detection Principles, In: Encyclopedia of Analytical Science, 2nd Edition, Academic Press, Vol: 3, 2005, pp. 48-56) (courtesy Elsevier Science Publishers).

Figure 4- Hybrid microflow LOV-based assembly equipped with an external reaction coil for the determination of ultratrace concentrations of nickel in brines following bead-injection preconcentration of the Ni-DMG chelate onto copolymeric divinylbenzene-co-n-vinylpyrrolidone beads and detection by electrothermal atomic absorption spectrometry. SP1 and SP2: Syringe pumps, HC: Holding coil, RC: Reaction coil (adapted from Ref. [78], courtesy Springer-Verlag).

Figure 1

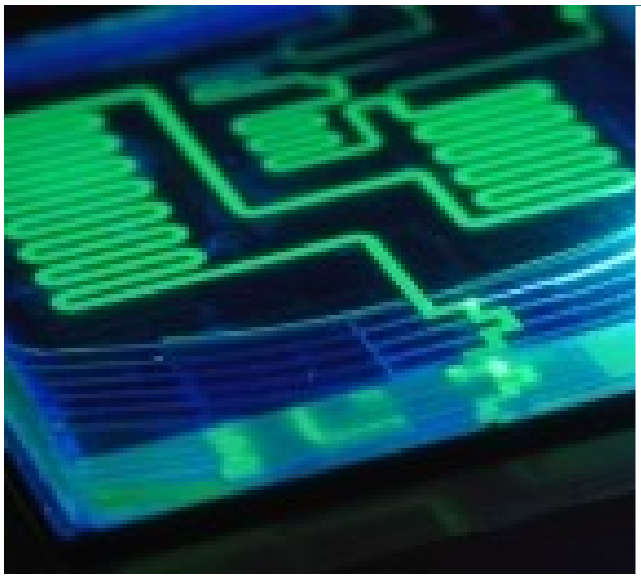

Figure 2

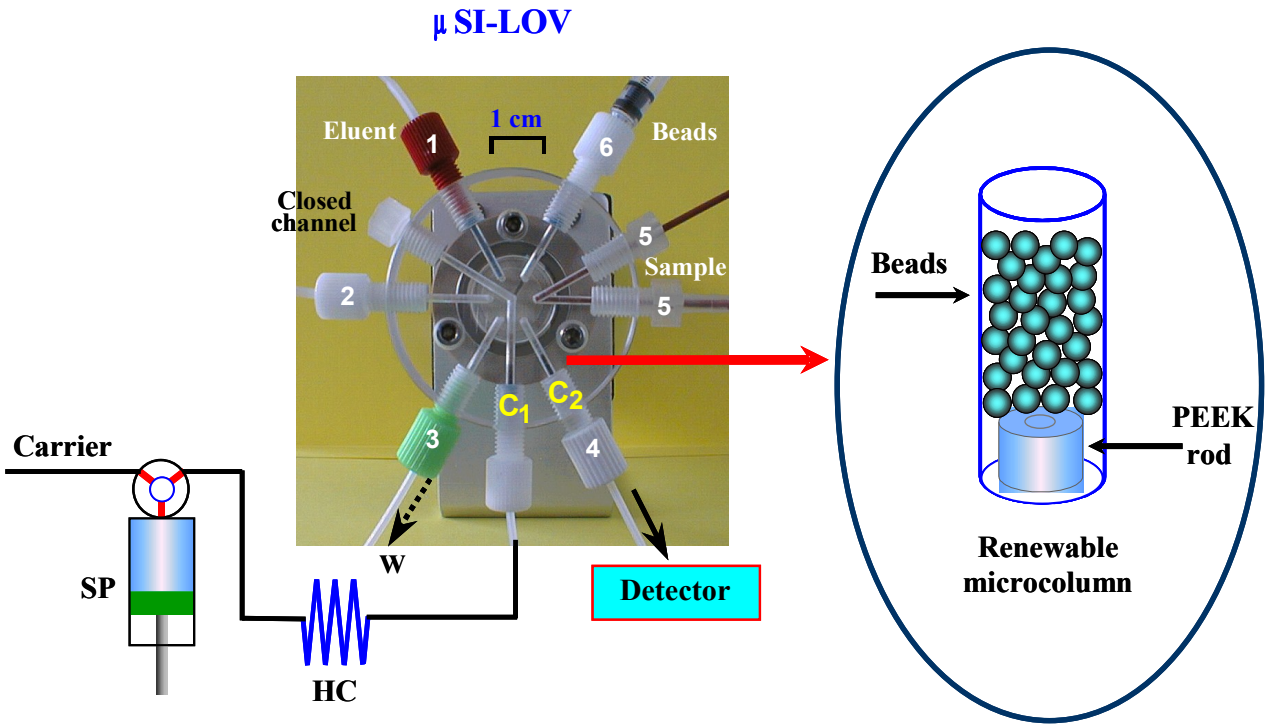
μ SI-LOV
Eluent
1 cm
Beads
Closed channel
Sample
Carrier
SP
W
HC
Detector
Beads
PEEK rod
Renewable microcolumn

Figure 3

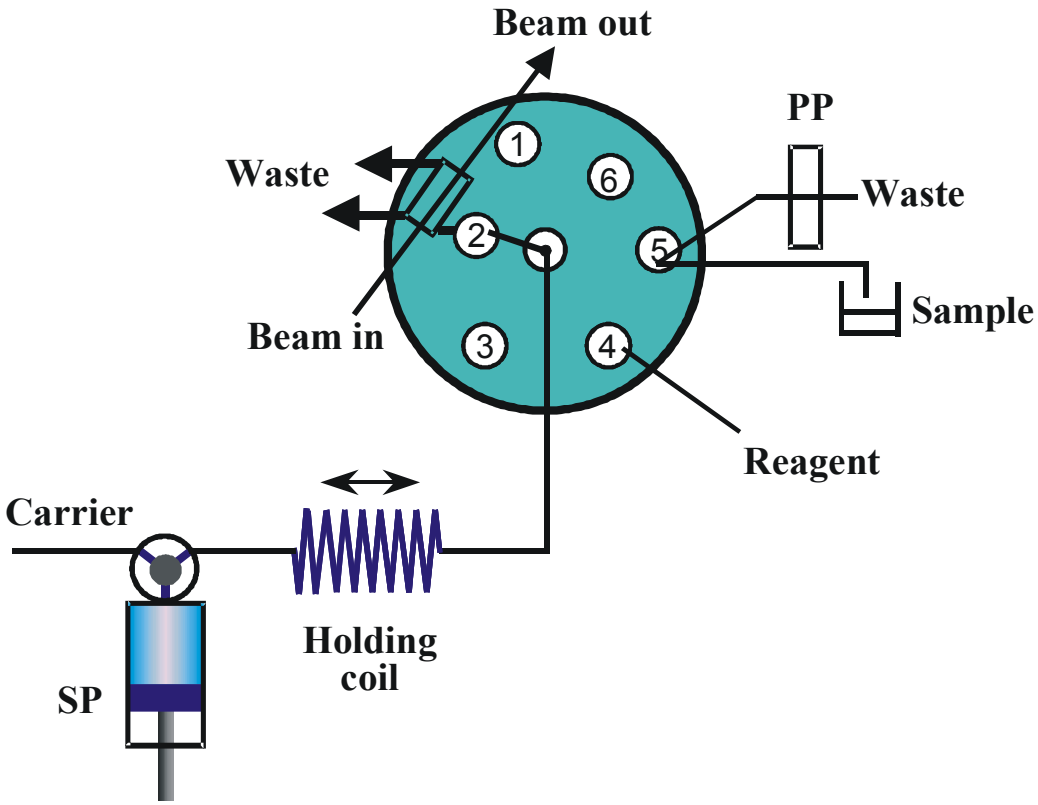
Beam out
PP
Waste
Waste
Sample
Beam in
Reagent
Carrier
Holding
coil
SP
1
2
3
4
5
6

Figure 4

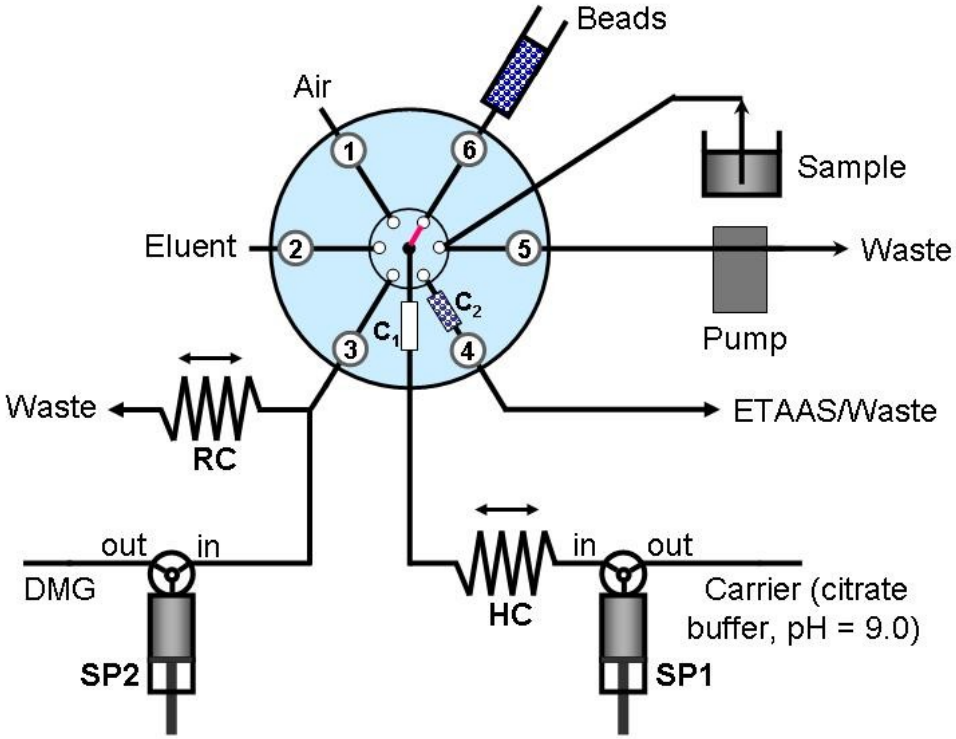
Beads
Air
Sample
Eluent
Waste
Pump
C_2
C_1
Waste
RC
ETAAS/Waste
out
in
DMG
SP2
HC
in
out
Carrier (citrate buffer, pH = 9.0)
SP1

Table 1- Relevant applications of LOV-based microfluidic analyzers for environmental assays

Analyte	Detection principle	On-line sample processing	Dynamic linear range	Detection limit	Precision	Matrix	Remarks/Features	Ref
Cr(VI)	ETAAS	Bead-injection (C_{18}-PS-DVB impregnated with DPC)	0.12-1.5 μg/L	30 ng/L	3.8 % (0.3 μg/L)	Seawater and hard tap waters	1) Overcoming the irreversible retention of the analyte by dissolution of both the reagent and complexed metal2)On-line pH adjustment to prevent interconversion between oxidation states	[60]
Ni(II), Bi(III)	ICP-MS	Bead-injection (ion-exchanger)	0.04-1.6 μg/L (Bi) 0.05-2.4 μg/L (Ni)	2 ng/L (Bi) 13 ng/L (Ni)	1.7 % (0.8 μg/L Bi) 2.9 % (0.8 μg/L Ni)	River sediment	1) Exploitation of a home-made direct injection high efficiency nebulizer 2) Implementation of a pre-elution step to minimize isobaric interferences by weakly retained metal species	[30]
Cd(II)	ETAAS	Derivatization + Bead-injection (PTFE/ C_{18}-PS-DVB)	0.05-1.0 μg/L	2 ng/L	3.0 % (0.5 μg/L)	Natural water and river sediment	1) Investigation of the feasibility of using hydrophobic materials in a renewable fashion 2)Tolerance to high salt concentrations with no need for matrix modifier	[83]
Sodium dodecyl-sulfate	SP	Solvent extraction	0.1-1.0 mg/L	0.01 mg/L	< 6% (0.5 mg/L)	Surface waters	1) Development of a Lab-at-valve approach 2)Implementation of optical fibers at the tip of the separation chamber	[67]
Nitrate, nitrite and ortho-phophospate	In-valve SP	On-column redox reaction	0.1-4.0 mg/L NO_3^- 0.03-4.0 mg/L NO_2^- 1.0-30 μg/L PO_4^{3-}	3.91 μg/L NO_3^- 4.53 μg/L NO_2^- 0.1 μg/L PO_4^{3-}	0.35 % NO_3^- 0.87 % NO_2^- 0.80 % PO_4^{3-}	Lake and tap water	1) Application of programmable flow to accommodate reactions with divergent kinetics 2) Bi-directional flow to minimize back-pressure effects in the Cd-foil filled microcolumn	[76]
Thiosulfate, chloride, nitrite, nitrate, citrate, fluoride, sulfate, phosphate, bicarbonate, acetate	Indirect-UV	CE separation	0.034-3.419 mM (chloride) 0.014-1.408 mM (sulfate)	1.56 μM (chloride) 0.8 μM (sulfate)	< 6.5 % (chloride) ≤ 3.5 % (sulfate)	Synthetic mixtures	1)Selection of various sample injection modes at will 2)Automated preparation, conditioning and reactivation of the capillary	[31]

Analyte	Detection principle	On-line sample processing	Dynamic linear range	Detection limit	Precision	Matrix	Remarks/Features	Ref
Cu(II)	In-valve SP	Derivatization	0.1-2.0 mg/L	0.05 mg/L	2.0 % (0.5 mg/L)	Wastewaters from electroplating industries	1) Use of air-segmentation for minimization of dispersion and improved mixing between zones 2) Application of the standard addition method to prevent multiplicative matrix interferences	[72]
Cr(III) and Cr(VI)	ETAAS	On-line reduction + bead-injection (chelating beads)	0.02-0.28 μg/L (Cr (III)) 0.035-0.40 μg/L (Cr (VI))	0.01μg/L (Cr (III)) 0.02 μg/L (Cr (VI))	< 2.5 % (0.2 μg/L)	Tap and surface waters and river sediment	1) Simultaneous processing of two sample aliquots 2) On-line Cr(VI) reduction under stopped-flow conditions exploiting an external reactor	[79]
Hg(II)	UV-SP	Hydride generation	10-315 μg/L	9μg/L	5 %	Synthetic samples	1) Critical comparison of performance of Venturi-type and expansion-type separators 2) Adaptable to in-field measurements	[25]
Cr(VI)	ETAAS (optional FAAS)	Fractionation + bead-injection (anion-exchanger)	0.02-0.6 ng	---	5.3 % (100 mg soil)	Agricultural soil	1)Application of dynamic fractionation with on-line processing of the extracts 2) Determination of the water and acid soluble fraction (readily available chromate)	[84]
Cd(II)	ETAAS	Derivatization + bead-injection (reversed-phase beads)	0.01-0.2 μg/L	1.7 ng/L	2.1% (0.05 μg/L)	River sediment	1) Mechanistical studies on the retention process 2)Use of C_{18}-chemically modified beads as universal media for precipitate collection	[58]
Ni(II)	ETAAS	Derivatization + bead-injection (co-polymeric beads)	0.2-2.0 μg/L	0.05 μg/L	4.8 % (0.8 μg/L)	Hard tap water and seawater	1)Application of co-polymeric sorbent with a balanced ratio of hydrophilic-lipophilic monomers 2) Increase of reaction time by using an external reactor clustered to the multiposition selection valve	[78]

Analyte	Detection principle	On-line sample processing	Dynamic linear range	Detection limit	Precision	Matrix	Remarks/Features	Ref
Cu(II) and Fe(III)	In-valve SP	Derivatization	0.1-2.0 mg/L Cu(II) 0.1-5.0 mg/L Fe(III)	50 µg/L Cu(II) 25 µg/L Fe(III)	2.0% (0.5 mg/L Cu(II)) 1.8 % (0.5 mg/L Fe(III))	Industrial wastewaters	1) Sequential determination of both metal ions in the same analyzer 2) Matching the composition of carrier with reagent solutions to minimize blank signals	[69]
NSAIDs	LC-UV	Bead-injection + chromatographic separations	0.4-40 µg/L	0.02-0.67 µ g/L	< 11 % (renewable mode)	Surface water, urban wastewater	1)Exploitation of a multisyringe flow network for preventing HPLC band broadening 2) Cost-effective approach as regards to commercial robotic systems	[33]
Cd(II)	VG-AFS	Derivatization + bead-injection separation	---	3.5 ng/L	1.6 % (0.1 µg/L)	River sediment	1) Co-precipitation of Cd(II) with lanthanum hydroxide 2) Development of a hybrid flow system for chemical vapour generation	[28]
Inorganic Arsenic	HG-AFS	Bead-injection (ion-exchanger) + post-column derivatization	0.05-2.0 µg/L	0.02 µg/L	< 6% (0.1 µg/L)	Tap, underground, lake and drinking water	1) Coupling of a multisyringe flow system for hydride generation following arsenic preconcentration 2) Implementation of various reactions with divergent kinetic demands	[29]
Chloride	In-valve SP	Displacement reaction	0-100 mg/L	45 µg/L	< 5% (50 mg/L)	Synthetic solutions	1) Extension of dynamic linear range by flow-reversal approaches 2) Careful consideration of the background absorbance of the reagent	[68]
Pb(II)	ETAAS	Bead-injection analysis (chelator)	1-4 ng	0.3 ng	1.9 % (2 ng)	Synthetic solutions	1)Use of reagent-loaded beads 2)Automated injection of matrix modifier for improvement of the pyrolysis step	[]

Acronyms: ETAAS: Electrothermal atomic absorption spectrometry, ICP-MS: Inductively coupled plasma mass spectrometry; CE: Capillary electrophoresis, SP: Spectrophotometry, FAAS: Flame atomic absorption spectrometry; LC: liquid chromatography, VG: vapour generation, HG: Hydride generation, AFS: atomic fluorescence spectrometry, NSAID: non-steroidal anti-inflammatory drugs, PTFE: polytetrafluoroethylene, PS-DVB: poly(styrene-divinylbenzene), DPC: 1,5-diphenylcarbazide

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