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Recent Developments in Flow Injection/Sequential Injection Liquid-Liquid Extraction for Atomic Spectrometric Determination of Metals and Metalloids

Aristidis N. Anthemidis¹ and Manuel Miró²

¹Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University, Thessaloniki, Greece

² Department of Chemistry, Faculty of Sciences, University of the Balearic Islands, Palma de Mallorca, Illes Balears, Spain

Abstract: This review aims to provide a critical overview of automated flow injection and sequential injection liquid-liquid extraction for preconcentration and/or separation of ultra-trace metal and metalloids species hyphenated with atomic spectrometric detection systems, including some new trends and applications in the subbranches of cloud point extraction (CPE), wetting film extraction (WFE), supported liquid membrane extraction (SLME), extraction chromatography (EChr), and liquid-phase microextraction (LPME) techniques. The analytical performance of flow-injection/sequential injection liquid-liquid extraction methods is markedly affected by the components of the flow network such as segmentor, extraction coil, and phase separator. Thus, an overall presentation of system components along with some novel strategies for interface with atomic spectrometers is discussed and exemplified with selected applications.

Keywords: Flow injection, sequential injection, liquid-liquid extraction, trace metal determinations, atomic spectrometry

INTRODUCTION

Atomic spectrometric techniques are widely employed for the determination of metallic species at trace and sub-trace-level concentrations. Flame atomic

Address correspondence to Aristidis N. Anthemidis, Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University, Thessaloniki 54124, Greece. E-mail: anthemid@chem.auth.gr

absorption spectrometry (FAAS) presents good selectivity and low cost operation while the dynamic range is typically within the mg L^{-1} range. Inductively coupled plasma atomic optical spectrometry (ICP-OES) has several advantages as simultaneous multi-element determination and low detection limits, which are typically better than those obtained by FAAS. Electrothermal atomic absorption spectrometry (ETAAS) as well as ICP-OES and inductively coupled plasma mass spectrometry (ICP-MS) are detection techniques inherently characterized by remarkable sensitivity (1–3). However, ETAAS signals are susceptible to sample matrix composition as well as to other operational factors (4), while in ICP-OES and ICP-MS both high contents of dissolved salts and spectral matrix interferences can produce undesirable analytical responses. According to the above remarks, it is obvious that despite recent advances in analytical instrumentation the most effective way to overcome or attenuate the above drawbacks is to separate analytes from the matrix components prior to the detection step, while at the same time, analyte preconcentration is taking place.

Solution handling is one of the most frequently performed laboratory tasks. It is also the most time-consuming and laborious one, as the solutions must be precisely metered, mixed, incubated, separated, and monitored in a reproducible way by atomic spectroscopy or by other detectors for quantification of the target analyte. Also, sample pretreatment still remains the bottleneck for a large number of analytical procedures and often the one that seriously hinders automation and miniaturization. The important role of flow injection (FI) techniques for automation, acceleration, and miniaturization of solution handling in sample pretreatment has been well demonstrated (5, 7).

During the last decades, the three generations of FI have played a dominant role in the automation of sample processing, which offers the figures of merit of minimized sample/reagent consumption and risk of sample contamination, fast analysis rate, and ease of hyphenation with various detection techniques (7). These characteristics have a profound impact on modern analytical sciences. It is also worth adding that the lab-on-valve (LOV) concept proposed by Ruzicka in 2000 (8) not only provided a more flexible approach for flow manifold design but also opened a promising avenue for miniaturization of the overall instrumental setup. This feature is especially important where rare and expensive samples/reagents are employed (9–12). So far, LOV, so-called the third generation of FI, has found wide applications in miniaturized sample pretreatment and quite a few publications have been located in the literature dedicated to automated separation and preconcentration of trace metal species (13, 14).

Due to the valuable advances in separation and quantification the conventional liquid-liquid extraction (LLE) is still the most widely used sample pretreatment technique in routine analyses for improvement of sensitivity and/or selectivity of analytical methods. In manual operation LLE procedures are usually very tedious and laborious and involve consumption of large amounts of hazardous organic solvents and reagents. In addition, they are prone to sample contamination, which precludes accurate determinations of trace-level

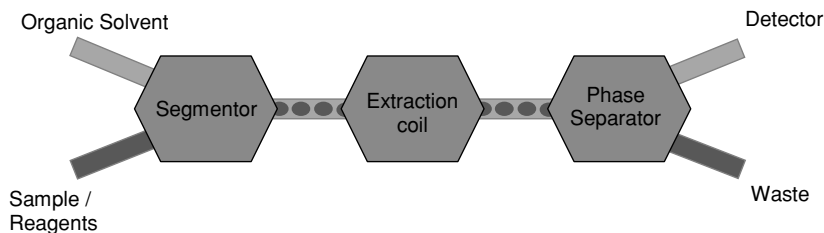


Figure 1. Schematic diagram of a typical FI manifold for on-line LLE.

concentrations of target species. The implementation of LLE in flow manifolds simulating the same sequences performed in batch procedures eliminates many of these drawbacks and contributes advantages inherent in automatic methods of analysis (15).

The introduction of flow injection liquid–liquid extraction (FI-LLE) as a mean for analyte preconcentration and/or separation in atomic spectrometry was first presented by Nord and Karlberg (16) in 1981. Since then, applications of such systems for routine analyses are rather limited compared to other on-line preconcentration systems as solid-phase extraction. This fact can be attributed to the complexity of the manifolds, which usually lack ruggedness and long-term stability as demanded in standard operational procedures (6). Further shortcomings are associated to the limitations on the aqueous to organic phase ratios that on-line phase separators can handle successfully (17) and difficulties in transportation of organic solvents using conventional FI devices. Organic solvents like isobutyl methyl ketone (IBMK), which is the most exploited in FAAS, cannot be reliably propelled for extended periods even with solvent-resistant pump tubes. Although displacement bottles have been introduced and successfully used by a vast number of researchers, considerable technical skill is required for proper manipulation (18). Besides, flow manifolds furnished with displacement bottles necessitate continuous operational maintenance because of the limited volume of organic solvent, which can be used without refilling the bottle.

The developments and advantages of FI and related techniques thereof for sample processing, including applications and considerations of LLE, have been discussed in some monographs (5, 7, 15, 19–21) and a number of comprehensive reviews (6, 22–30).

This review presents and critically discusses the state-of-the-art developments of FI/SI LLE for preconcentration and/or separation of ultra-trace metal and metalloid species hyphenated with atomic spectrometric detection systems, including some new trends and applications in the subbranches of cloud point extraction (CPE), wetting film extraction (WFE), supported liquid membrane extraction (SLME), extraction chromatography (EChr), and single-drop microextraction techniques.

FUNDAMENTALS OF ON-LINE LLE FOR TRACE-METAL DETERMINATIONS

Conventional FI-LLE comprises distribution of target metal species between two immiscible liquid phases (usually aqueous and organic). Mass transfer between both phases takes place when they are brought into contact to form a regularly segmented stream that is subsequently separated downstream.

A typical FI manifold (Figure 1) for on-line LLE consists of three essential parts: a segmentor (e.g., a confluence point for the phases); an extraction coil, in which the analyte is transferred from one phase to the other; and a phase separator for continuous separation of the segmented stream into two parts, at least one of them containing a pure phase.

In classical FI systems, aqueous sample solutions are usually introduced discretely into a continuous aqueous carrier, which is merged downstream with another aqueous stream containing a complexing reagent. The resulting aqueous stream bearing the in-line generated non-charged metal chelate is then segmented with an organic immiscible solvent stream at the segmentor mixing point. Following chelate extraction, the segments of aqueous and organic phase are separated in the phase separator, whereupon the derivatized analyte in the pure receiving phase is determined using a continuously or discretely operating atomic spectrometer.

Each particular operation in FI-LLE is important for obtaining high sensitivity with the lowest sample zone broadening. Kuban overviewed basic extraction principles of FI-LLE as well as its theoretical aspects and applications (22, 31). In the following, a brief discussion of FI-LLE components is given:

1. Segmentor is the unit providing alternate and regular segments of the two immiscible phases in a single channel, while segmentation is the result of two different mechanisms. The formation of droplets of one phase into the continuous flow of the other phase, immiscible with the first, and the so-called ripple process, which is the result of the destruction of the thick layer of one of the phases that is formed at high flow rates on the walls of the segmentor. The characteristics of segmentors influence the reproducibility and the size of segments. Though segment size might not affect significantly the extraction efficiency of a fast extraction process, it could theoretically influence the efficiency of slow extraction systems. Yet, the length of the segments (organic and aqueous) cannot be reduced indefinitely. Nord and Karlberg estimated experimentally the lower limit of organic phase segments to be 1.5 times the internal diameter of the extraction coil tube (32). The extraction efficiency decreases drastically if the droplets of organic phase become too small to form a continuous film on the tubing wall of the segmentor. Also, the segmentation may be unstable due to coalescence of very small segments during transport through the extraction coil.

Several segmentor configurations (22, 31) with variable efficiencies have been described in the literature. Y- or T-pieces made of homogeneous materials (e.g., fluoroplastics, stainless steel, or glass) or combination of hydrophobic and hydrophilic materials are usually utilized. An alternative type of segmentor, the coaxial type (falling drop), which operates on the basis of differences of gravity or density, has been presented in order to overcome some of the disadvantages of the other types (33). Very precise and repeatable volumes of one phase can be introduced into a continuous flow of the other phase using a rotary valve (34). In this case, the segment length is being controlled by the preselected volume of the injector loop.

2. The extraction coil is the unit in which extraction takes place but to some extent extraction can also be done in the segmentor and phase separator. The metal chelates are transferred from a homogeneous aqueous phase into segments of the immiscible organic phase via segment interface. However, the thin organic film formed onto the inner walls of the extraction coil is proven to contribute significantly to the extraction yield (33). The LLE of neutral chelates generally depends on the sample residence time within the extraction coil, which is affected by the extraction coil dimensions (length and inner diameter) and flow rate. Degree of extraction increases with decreasing the inner diameter of the extraction tube. The extraction coil should be made sufficiently long to prevent transfer kinetics being the limiting factor in extraction efficiency. Yet, deterioration of enrichment factors is observed when employing unduly long extraction tubes.
3. The phase separator is the crucial component of on-line continuous LLE systems. The phase separation process involves a partitioning of the segmented phases and delivery of metal containing organic phase to the detection system. In most separators, the two-phase system cannot be, however, splitted totally into two pure individual phases. The targets of phase separators in flow manifolds are as follows: (i) continuous separation of the phases, (ii) recovery of as much as possible of organic phase, (iii) acceptance of a wide range of flow rate ratios (Q_{aq}/Q_{or}) for immiscible phases, and (iv) handling of minimal volumes of a vast variety of organic solvents. Under optimized conditions, on-line phase separation efficiency ranges from 70 to 90% (20) while the preconcentration factor is limited by the moderate Q_{aq}/Q_{or} ratio that phase separators can handle successfully.

Phase separators have been studied in detailed concerning design, construction, and operation performance (18, 35–39) prior to atomic spectrometric detection. Those currently used in FI/SI systems can be categorized as microporous membrane types that use a hydrophobic (and/or hydrophilic) membrane to exclude one or both phases from the segmented stream (40, 41), affinity (with heterogeneous material) separators that are working on the principle of affinity difference between the phases and the materials of the separator as well

as the density difference of the two phases (33), and gravitational (or density) separators based solely on differences in phase density (18, 36, 39, 42–44).

The selection of an appropriate membrane material for the membrane-type separator depends on flow rate of organic phase, Q_{aq}/Q_{or} ratio, physical and chemical properties of solvent, separator design, durability of membrane, and sample throughput. Polytetrafluoroethylene (PTFE) membranes of different pore size (typically 0.7 to 0.9 μm) are preferred over cellulose acetate or cellulose nitrate membranes because they are more hydrophobic, durable, and commercially available in a wide choice. The main drawbacks of membrane-type phase separators are the short lifetime of the membrane due to fouling and leakage of minute amounts of aqueous phase whenever high Q_{aq}/Q_{or} ratios and high segmented (total) flow rate is applied. However, membrane phase separators cause lower dispersion than the affinity or density counterparts.

In order to improve the ruggedness of continuous LLE systems, Tao and Fang (37) proposed a membrane-less design for phase separator involving the use of a two-part conical cavity gravitational phase separator. The conical shape of the separation cavity combined with the differences in density of the two phases and hydrophobic/hydrophilic nature of the cavity sections permitted the continuous separation of two phases with no need for membrane. The separator has been successfully used for Ni and Pb determination by ETAAS and FAAS, respectively (37, 45).

Nielsen and Hansen markedly improved the analytical performance of gravitational separators through a detailed investigation on the design and dimensions. The proposed gravitational phase separator consisted of two blocks. The upper block, which is fabricated from PTFE, is shaped to contain a conical cavity (55 μL) and furnished with a single outlet for the separated phase, while the lower block, machined from stainless steel, is provided with an inlet and an outlet for the combined reaction mixture and the waste flow, respectively. The unique feature of the phase separator is that, despite its simple construction, it has a reliable long-term operational lifetime, as demonstrated for FI/SI-LLE of Cr and determination by ETAAS (46, 47). However, the reported design is only effective for separation of a low-density phase from a mixture stream. In order to foster the separation of both low-density and high-density phases, and hence allow both solvent extraction and back extraction, a novel dual-conical gravitational phase separator was designed by Wang and Hansen (18) and employed for cadmium determination via complexation with ammonium pyrrolidine dithiocarbamate (APDC) by SI solvent extraction-back extraction coupled to ETAAS detection. A schematic illustration of the SI-LLE manifold and phase separator fabricated from polyetheretherketone (PEEK) is shown in Figure 2. The separating chamber of approximately 140 μL is composed of two conical cavities of identical volumes. The extraction mixture is directed into the middle region of the separating chamber where the phase separation takes place. By controlling the outflow rates, the biconical chamber effectively facilitates the separation of both organic and aqueous phases. Besides use with

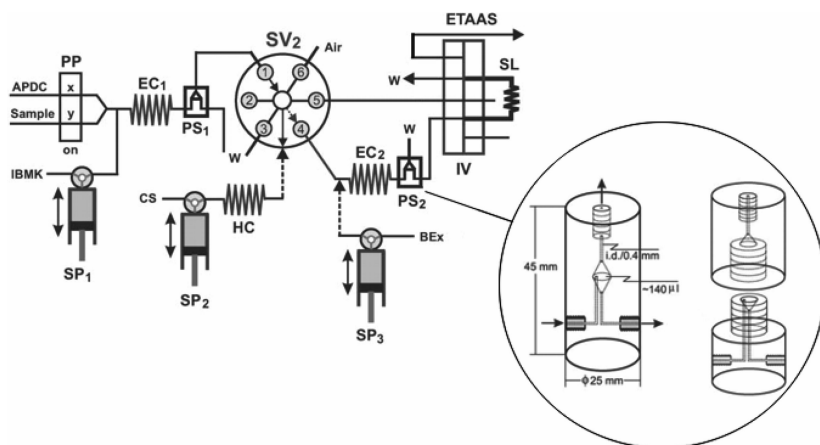


Figure 2. SI LLE back-extraction manifold hyphenated to ETAAS for trace-metal determination. SP₁, SP₂ and SP₃, syringe pumps; PP, peristaltic pump; EC₁ and EC₂, extraction coils; PS₁ and PS₂, dual-conical gravitational phase separators (not scaled); SV₂, 6-port selection valve; HC, holding coil; SL, sample loop; IV, 2-position injection valve; CS, carrier solution; BEx, aqueous back-extractant solution; W, waste (18).

ETAAS, it was proven suitable for coupling to ICP instruments (38), offering an interesting and easy alternative approach to other complicated interface methods described in the literature when using organic extractants prior to ICP detection. Back-extraction was in this instance facilitated via an exchange reaction involving the use of Hg(II) ions (18).

Anthemidis et al. (17, 39) developed a novel gravitational phase separator with improved tolerance to high Q_{aq}/Q_{or} ratios for FI-LLE and determination of trace elements as hyphenated to either ETAAS or FAAS. The performance of the system was evaluated for ultra-trace cadmium determination using IBMK as extractant. The dedicated separator is composed of a cylindrical glass chamber with volume capacity of 11.7 mL and PTFE push-fit connections at the two ends. The above connectors contain internal conical cavities of ca. 30 μL in order to facilitate the separation of the organic solvent. The upper PTFE connector has a single outlet (0.3 mm id) for the separated organic phase, while the lower one is drilled to accommodate the inlet (1.0 mm id) for the segmented stream and an outlet (1.0 mm id) for aqueous waste. Thus, the low-density organic phase (i.e., IBMK) was raised to the upper region, while the aqueous phase was collected in the lower one due to the different phase densities. The above configuration is able to admit Q_{aq}/Q_{or} ratios up to 40 and segmented flow rate $\leq 18.5 \text{ mL min}^{-1}$, whereby high preconcentration factors and extraction efficiencies are to be attained. The main advantages of the proposed phase separator are: (i) Ability to tailor enrichment factors to the analysis needs by selection of Q_{aq}/Q_{or} ratios, (ii) collection of concentrate (organic) phase volumes at will by time-based

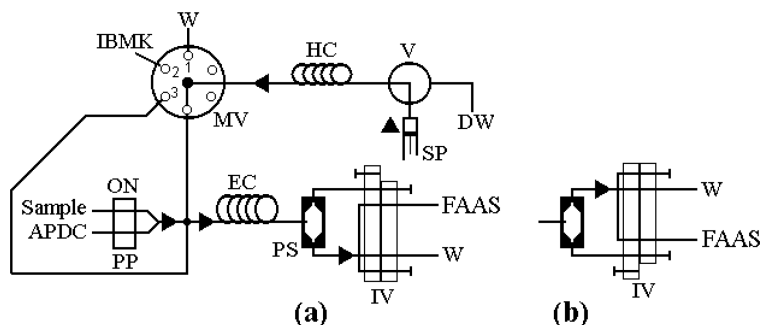


Figure 3. SI-LLE system coupled to FAAS for metal determination. MV, multiposition valve; SP, syringe pump; V, syringe pump valve; PP, peristaltic pump; IV, injection valve; EC, extraction coil; HC, holding coil; PS, microphase separator; DW, distilled water; W, waste. (42).

control and as opposed to membrane-type separations, (iii) unlimited lifetime, (iv) separation efficiencies of 100%, and (iv) simple manufacture with no need for expendable parts (e.g., membranes). However, it is merely applicable to low-density organic solvents and considerable technical skill is required for smooth operation.

A downscaled gravitational phase separator with a total inner volume of 900 μL has been recently designed and implemented within an SI-LLE-FAAS assembly as shown in Figure 3a (42). The extraction coil enters into the microseparator horizontally and fosters fast separation and collection of organic phase free of aqueous phase regardless of the density of the organic solvent (Fig. 3a, b).

MANIFOLD AND INTERFACES

Despite the various attempts at improving on-line LLE systems in atomic spectrometry applications as described above, the number of publications remains rather low as compared to sorptive preconcentration procedures. Such a situation seems to be the result of difficulties associated with organic solvent manipulation, both in maintaining stable flows using conventional FI fluid delivery equipment, as well as in providing long-term efficient and reliable phase separation.

Various FI/SI-LLE arrangements (17, 18, 38, 39, 42, 46–49) of different complexity are described in the literature as hyphenated with atomic spectrometric detection systems, namely, FAAS, ETAAS, ICP-OES, and ICP-MS. The interface between FI/SI-LLE manifolds and continuously operating detectors, such as FAAS, ICP-OES, and ICP-MS, requires the incorporation of conventional rotary valves for injection of metal containing organic phase. As

a consequence of the programmable discontinuous flow nature of SI, the in-line coupling of SI-LLE with ETAAS detection is relatively simple, because of the discrete, noncontinuous operation of the detector. The organic extract is readily directed to the graphite tube platform via air-segmentation. Yet, it has been proven that organic solvents tend to distribute along the length of the tube because of their lower surface tension and good wetting ability, thus resulting in deterioration of sensitivity and precision of measurement (50). To take advantage of the high surface tension of water, it is therefore beneficial, as opposed to FAAS detection, to back-extract the analytes from the organic phase into an aqueous phase prior to ETAAS quantification as described by Wang and Hansen (18).

The on-line coupling of FI-LLE with ETAAS is, however, feasible as demonstrated by Bäckström and Danielsson for the determination of Cd, Co, Cu, Fe, Ni, and Pb in aqueous solutions (50). The method involved a double extraction procedure for enhancement of extraction efficiency followed by a back-extraction step. The final extract was collected in a 23- μL sample loop of an injector and delivered directly into the atomizer by means of a programmable sampling arm using positive air displacement. The LLE procedure was performed in parallel with the furnace temperature program in order to improve sample throughput. The same authors' group developed further the on-line LLE system for determination of aluminum in natural waters and also for evaluation of the lipophilicity of metal complexes via the aluminum partition coefficient in *n*-octanol/aqueous systems (51, 52).

Tao and Fang (37, 53) devised an FI-LLE-ETAAS assembly for Ni determination, which is characterized by a dedicated gravitational phase separator (see above) and the use of PTFE pump tubes for organic solvent propulsion. Although the manifold is simple, the proposed flow network needed to be operated under extreme conditions (e.g., sample flow rates $> 5.5 \text{ mL min}^{-1}$ and $Q_{\text{aq}}/Q_{\text{or}}$ ratios > 25) for achieving appropriate preconcentration factors. The same group developed an improved dual-stage preconcentration system based on FI on-line ion-exchange and liquid-liquid extraction for Pb determination by FAAS. A column packed with Amberlite IRC-718 cation exchanger is incorporated into an FI-LLE manifold. The analyte is retained on the column and eluted by $1 \text{ mol L}^{-1} \text{ HNO}_3$. The eluate is subsequently merged with potassium iodide and tetrabutylammonium bromide, whereupon the ion-paired metal species is extracted in-line into IBMK and separated by a gravity phase separator similar to the previous one and introduced into the FAAS (45).

Despite the recent instrumental advances in fast sequential FAAS/ETAAS or continuum source-AAS, truly multielemental determinations are effected by coupling of FI/SI-LLE with ICP-OES or ICP-MS. When ICP-MS is used as the detection device, direct introduction of organic sample solutions is, in fact, prohibitive as a consequence of spectroscopic and nonspectroscopic interfering effects and the deposition of carbon on the sampler cone of the MS interface, which drastically affects the sensitivity and the stability of the signals. Since

most samples are aqueous, the preconcentration procedures therefore require a combination of extraction and back-extraction. The preconcentration efficiency might be controlled by adjusting the aqueous/organic and organic/aqueous volume ratios in the two extraction steps, respectively. In the back-extraction step itself, several operational chemical parameters can be utilized to obtain optimal conditions, such as the pH of the aqueous solution and the addition of a suitable stripping agent. This has been nicely exemplified by Wang and Hansen (38) for SI-LLE and ICP-MS determination of Cu and Pb. The back-extraction procedure was accomplished using Pd(II) as stripping agent to accelerate the kinetically slow back-extraction of elements into acidified aqueous solutions (38). However, few examples are found in the literature describing the coupling of FI/SI-LLE/back extraction with ICP-MS detection, which is attributed to the sophistication of the flow systems, the difficulties associated to in-line manipulation of organic solvents and a lack of robustness of the components of FI/SI-LLE set-ups (27, 54).

UNCONVENTIONAL FI/SI-LLE METHODS

Unconventional LLE schemes herein discussed are classified as cloud point extraction (CPE), wetting film extraction (WFE), supported liquid membrane extraction (SLME), and extraction chromatographic (EChr) methods.

Cloud Point Extraction (CPE)

Following up the first approaches of CPE presented by Watanabe and Tanaka (55) as a new promising alternative to LLE for metal preconcentration and/or separation, the number of annually appearing publications has been continually growing concerning either off-line or on-line procedures. The principles and theoretical aspects as well as relevant applications regarding the use of micelle-mediated separation have been well documented in various interesting reviews (56–58). The CPE procedure is based on the phase separation that occurs in aqueous solutions of nonionic surfactants when heated above the so-called cloud point temperature. At this point the initial solution separates into a surfactant-rich phase of small volume containing the metal trapped by organized structures and a bulk aqueous phase (57).

Ortega et al. (59, 60) were the first in coupling CPE on-line with ICP-OES for determination of rare earth elements in urine. The methodology is based on in-line metal complexation with 2-(5-bromo-2-pyridylazo)-5-diethylamino-phenol in the presence of non-ionic micelles of PONPE-7.5. The micellar system containing the complex was thermostated in-line in order to promote phase separation, and the surfactant-rich phase was retained in a microcolumn

packed with cotton. The surfactant-rich phase was eluted with nitric acid directly in the nebulizer of the plasma.

Nan et al. (61) developed an FI-CPE procedure for ETAAS determination of trace Pb in biological samples. The procedure involved the in-line formation of the analyte complex with ammonium pyrrolidine dithiocarbamate (APDC), which is subsequently mixed with a surfactant solution of Triton X-114. It was found that a polar sorbent, like silica gel, packed in microcolumn performs well for the retention of the analyte-entrapped surfactant micelles because of the hydrophilic and polar nature of the outer sphere of the micelles. Experimental operations such as heating or addition of electrolytes and filtration or centrifugation for phase separation that were required in conventional CPE were not required. Using a similar system configuration and O,O-diethyldithiophosphate as chelating reagent, Bai and Fan (62) described an FI micelle-mediated alternative method for Pb determination. However, a threefold increase of sample loading was required to attain a similar enrichment factor than that achieved by Nan et al. (61).

Li et al. (63) have recently reported a semiautomatic CPE procedure for speciation of inorganic Sb species as detected by electrothermal vaporization (ETV)-ICP-OES. The method involves at-line selective complexation of Sb(III) with APDC at pH 5.5 prior to in-line preconcentration of organized entities in a cotton microcolumn. Total antimony determination is realized after reduction of Sb(V) to Sb(III) by L-cysteine. Identical flow-through configuration has been utilized by Yamini et al. (64) for multielement (namely, Cd, Fe, Ni, Co, Cr, Mn) enrichment prior to ICP-OES measurements. However, maximum enrichment factors of 97 are reported as compared to 872 for Sb(III) in Li's work (63).

Advantageous features that promoted on-line applications of micelle-mediated extraction might be summarized as follows: (i) Preconcentration factors are comparable to those of other sample processing schemes, and might be modified on demand by varying the amount of surfactant; (ii) commercially available surfactants are environmentally friendly and cost effective; and, finally, (iii) the amount of organic solvents used are minimal as compared to those employed in LLE (56). Thus, on-line CPE should be regarded as an appealing "Green Chemistry" method.

Wetting Film Extraction (WFE)

The most critical aspects of FI/SI-LLE systems are phase segmentation and separation because of their significant dependence upon reproducibility and reliability of analytical procedures and dispersion of the sample zone as well. Numerous efforts have been made to eliminate the need for segmentation and phase separation. To this end, the so-called wetting film extraction (WFE) concept was proposed in 1996 (65). It is based on the formation of a coating on the inner walls of a PTFE extraction coil with a thin layer of organic phase

as a result of the hydrophobic interactions between the solvent and reactor, which delay the organic phase with respect to the aqueous solutions. The enhanced aqueous/organic phase volume ratio in comparison with classical FI-LLE renders high enrichment factors. This film is also responsible for high extraction efficiencies, avoiding the axial dispersion of the analyte occurring in segmented FI-LLE. Once the metal chelate species has been preconcentrated, a small plug of a suitable solvent/back-extraction solution is pumped into the coated reactor to strip out the isolated analyte, which is thereupon delivered to the atomic spectrometer for quantification. The organic film is removed after each analysis cycle, whereby the WFE approach is free from carryover effects.

The theoretical aspects as well as fundamental stages of the renewable-phase WFE technique have been well presented by Miró et al. in a recent review (27). The majority of proposed manifolds for this sample processing approach have been related to SI systems furnished with either peristaltic or syringe pumps. Chen et al. (66), however, implemented WFE in a time-based FI system for determination of Cu by FAAS. The authors emphasized the simplicity of the flow manifold, which was constructed from a software-controlled rotary injection valve and two peristaltic pumps. Due to the very low elution flow rate in WFE, which is not compatible with the nebulizer's free uptake flow rate of FAAS, the authors introduced a special sequence in the FI program for fast aspiration of the discrete eluate zone into FAAS. Thus, both FI-WFE and FAAS were able to work at independently optimized flow rates, which rendered improved sensitivity. Anthemidis and Adam extended the combination of FI-WFE with FAAS for cadmium determination using a thin film of *n*-isobutyl ketone (DIBK) on the inner wall of a PTFE extraction coil and DDTTC as complexing agent in a simple manifold (67).

The WFE approach has been also proven suitable for metal speciation analysis by selective formation and extraction of chelates or ion-paired complexes prior to measurement. Christian and coworkers proposed an SI-WFE method for speciation of Cr(VI) and Cr(III) (68), which could be readily adapted to any atomic spectrometric detection system.

Miró et al. (69) extended the SI-WFE approach to in-line separation of radionuclides, namely ^{90}Sr , in environmental samples and dairy products. A lipophilic macrocycle host was incorporated in the film-forming phase for selective extraction yet iterative backward-forward flows were essential for improved extraction efficiency. In comparison to common extraction chromatographic sorbents used in batch-wise or flow-based radionuclide separations (see below), SI-WFE offers minimum expenses of the costly crown ethers, expedition of the operational sequence, and high tolerance to concomitant irreversible interferences as a result of the renewable nature of the extractant phase. The coupling of SI-WFE/back-extraction with ICP-MS is actually a promising approach for determination of radionuclides at environmental levels.

Membrane Extraction

The role of extractive membranes for sample processing in various analytical arenas has been reviewed by different authors (70–74). The theory of mass transfer of analyte species from a sample donor solution to an acceptor stream via a membrane phase has also been discussed in detail (75). Two different membrane extraction techniques should be differentiated: The so-called polymeric membrane extraction where a nonporous membrane (e.g., polydimethylsiloxane or latex) separates the donor and acceptor solutions and the so-called supported liquid membrane extraction (SLME) where a microporous hydrophobic polymeric membrane (e.g., PTFE, polypropylene) is impregnated by an organic solvent, which is held within the pores by capillary forces. Typical solvents in this context are di-hexyl ether, tri-octyl phosphate, kerosene, and *n*-undecane. SLME extraction can be considered as chemically analogous to an LLE from an aqueous sample into an organic solvent followed by a back-extraction from the organic phase to another aqueous phase. The most important advantages of SLME as compared with FI-LLE are the low consumption of organic solvent, ease of automation, improved selectivity, and modulation of concentration enrichment by halting the acceptor solution (74). Although SLME applications have been mostly directed to the separation of ionizable organic analytes with octanol-water partition coefficients close to 10^3 (72), extension to metal ion species is feasible via carrier-mediated SLME. It is based on the solubilization of ion-pairing, hydrogen-bonding, or chelating reagents, e.g., methyltrioctylammonium chloride, tri-octylphosphine oxide, and di-2-ethylhexylphosphoric acid (DEHPA), respectively, into the membrane phase. As a result, neutral, extractable species are formed at the donor-membrane interface, which are swept irreversibly into the receiver solution after partitioning in the liquid membrane (71). An important limitation of SLME for application in flow systems concerns with the diffusional resistance to mass transfer through the membrane phase, which leads to poor sensitivity (72, 76). This could be alleviated to some extent, exploiting capillary-type configurations with a single perfused channel, so that extraction and stripping procedures are made sequentially at the same liquid-membrane interface (77). Another well-known pitfall is the short-term stability of liquid membranes, whereby regeneration protocols should be devised.

Several papers illustrating the advantages of FI for preconcentration of metal ions by SLME have been reported in the literature. Using 8-hydroxyquinoline as complexing reagent in the donor phase and diethylenetriaminepentaacetic acid (DTPA) as stagnant acceptor solution, extraction efficiencies up to 70% were obtained for Cu (78). In the same work, a more satisfactorily SLME assembly was proposed for separation of Cu, Cd, Co, Ni, and Zn as thiocyanate complexes, using a PTFE membrane impregnated in di-*n*-hexyl ether containing the anionic exchanger Aliquat 336 and a DTPA acceptor modified with the membrane additive. Atomic absorption spectrometry, either FAAS or ETAAS, was used for at-line determination of metal species in

the extracts. Enrichment factors of up to 500-fold were obtained. An automated system for trace metal (Al, Cd, and Cu) determination using four parallel PTFE membranes impregnated with 40% (w/w) DEHPA was developed by Malcus et al. (79) for high-throughput analysis. DEHPA was extensively used by the same authors' group as a ligand in liquid membranes for enrichment of Cu, Cd, and Pb in river water (80) and Pb in urine (81), prior to at-line analysis of the acceptor solution by ETAAS and FAAS. Mathiasson and coworkers (82) extended the combination of SLME with ETAAS for chromium speciation. The method involves the selective extraction and enrichment of anionic Cr(VI) and cationic Cr(III) species in two serially connected SLM units. Methyltri-caprylammonium chloride (Aliquat) and DEHPA were used, respectively, as selective extractants dissolved in the liquid membrane. Extraction efficiencies for Cr(III) and Cr(VI) under the optimized experimental conditions were 90 and 40%, respectively. Romero and Jönsson (83) proposed a highly selective SLM system using a liquid membrane bearing 1,10-dibenzyl-1,10-diaza-18-crown-6 and oleic acid in di-*n*-hexyl ether to concentrate free copper ions from natural waters. Initial experiments were performed to determine whether extraction of Cu²⁺ occurs across a liquid membrane that contains 40% (w/w) DEHPA in kerosene, as reported by earlier researchers for extraction of several metals in acidic milieu (80, 81). However, it was proven that DEHPA was promptly dissolved in the donor stream when increasing sample pH to 6–8.

Parthasarathy et al. (84) proposed the use of hollow fibers with small lumen as the supported liquid membrane (HF-SLM) instead of a flat sheet SLM for at-line determination of Cu, Pb, and Cd with atomic spectrometry. Experimental results revealed longer stabilities of the capillary-type membranes as compared with the flat-type counterparts.

Extraction Chromatography (EChr)

Extraction chromatography (EChr) is a sample processing technique utilized for isolation of radioisotopes from inactive matrix components and interfering radioactive fission products. In EChr, selective chelating agents or macrocyclic ionophores are dissolved in an organic solvent (e.g., octanol) and immobilized physically on porous polymeric resins. EChr separations in an FI/SI-mode should be viewed as an excellent alternative to manual extraction aiming to reduce drastically the analysis time while preventing potential health risks due to manipulation of radioactive samples. FI/SI-EChr involves the incorporation of a packed column bearing the selective reagent within the flow manifold prior to the detection system. Grate and coworkers (85) highlighted the potential of SI-EChr as a front-end to ICP-MS for actinide (e.g., Am, Cm, Pu, Th, Np, and U) isotopic measurements in nuclear wastewaters. Enhanced resolution of extracted actinide species may be accomplished in SI-EChr by applying multiple elution approaches. By proper selection of acid and complexing

eluent placed at the peripheral ports of the selection valve, the discrimination of actinides in different valence state groups, as well as the selective Pu and Th recovery, via on-column redox reactions and HF/HCl elution, respectively, was shown to be feasible (86). Further, isobaric, polyatomic, and spectral interferences encountered in the ICP-MS determination of actinides may be addressed by on-line hyphenation of two EChr-columns in series (87) or by careful choice of chemicals for reductive sample treatment prior to analysis (85). The versatility of SI has been utilized for application of automated sorbent renewable approaches (so-called bead injection protocols) regardless of the detection system (88), aiming at providing fresh sorptive columns for each sample, thus eliminating analyte carryover problems between consecutive assays and the loss of retention capability owing to irreversible extracted species. Although initial interest in SI/FI-EChr was focused on the analysis of highly radioactive samples, such as nuclear waste or reactor samples, recent publications in the field demonstrated that SI/FI-EChr does also possess potential for monitoring isotopes in radio-bioassays (89, 90) and environmental samples at a global fallout level (91, 92) after appropriate sample pretreatment.

LIQUID PHASE MICROEXTRACTION TECHNIQUES

The first attempt to miniaturize LLE in a flowing stream assembly was carried out by Liu and Dasgupta (93) in 1996. The authors reported a novel flow-through drop-in-drop microextraction system where a small drop ($\sim 1.3 \mu\text{L}$) of a water-immiscible organic solvent was immersed into a larger flowing aqueous drop to accomplish the extraction of ion-paired species. Further research led to the development of the so-called single-drop microextraction (SDME) where a droplet of organic solvent was suspended at the tip of a microsyringe needle and immersed into the aqueous sample. Psillakis and Kalogerakis (94) and recently Xu et al. (95) overviewed the basic extraction principles of SDME as well as its latest developments and applications.

The implementation of SDME in a continuous flow microextraction mode (CF-SDME) was firstly described by Liu and Lee (96), as illustrated in Figure 4. In a $500\text{-}\mu\text{L}$ glass chamber, an organic drop ($1\text{--}5 \mu\text{L}$) is held at the outlet tip of PEEK connecting tubing, which is immersed in an aqueous sample solution flowing at ca. $50 \mu\text{L min}^{-1}$. CF-SDME differs from other SDME approaches in the fact that the solvent is continuously surrounded by fresh aqueous solution, thus leading to improved extraction efficiencies (95).

Hitherto, the majority of reported papers on SDME have been focused on preconcentration of organic pollutants. Some efforts have been, however, devoted to metal extraction using SDME and at-line atomic spectrometric detection (97–99). Xia et al. (97) proposed for the first time the combination of CF-SDME with ETV-ICP-MS for determination of trace-level concentrations of Be, Co, Pd, and Cd in biological matrices. In this case, benzoylacetone was

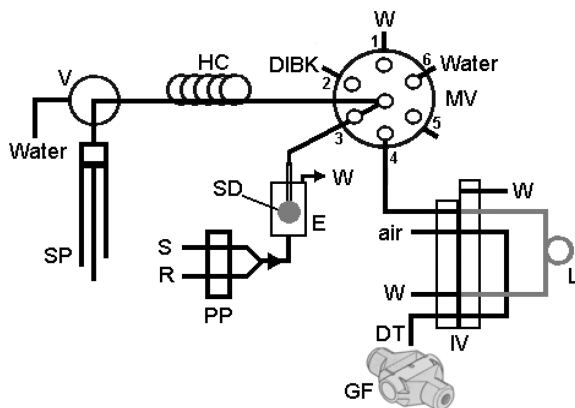


Figure 4. Schematic illustration of an SI-SDME manifold coupled to ETAAS for cadmium determination. S, sample; R, DDPA solution; W, waste; PP, peristaltic pump; SP, syringe pump; MV, multi-position valve; IV, injection valve in “load” position; V, valve in “out” position; HC, holding coil; L, loop; E, micro-extraction cell; SD, solvent droplet; DT, delivery tube; GF, graphite furnace ETAAS.

employed as both extractant and chemical modifier for improved selectivity and enrichment factors. The same authors’ group presented the assistance of 8-hydroxyquinoline as additive in a chloroform drop for speciation analysis of aluminium. Thus, free Al (namely, Al^{3+} , AlOH^{2+} , and $\text{Al}(\text{OH})_2^+$) could be quantitatively extracted while non-labile Al chelates remained in the sample solution (98).

Recently, Anthemidis et al. (100) developed a completed automatic SDME method performing on-line metal preconcentration and/or separation using sequential injection analysis coupled with ETAAS. The micro-drop of di-isobutyl ketone (DIBK) ($60 \mu\text{L}$) is generated and suspended at a glass capillary tip into the flow-through micro extraction cell (E) as illustrated in Figure 4. As the fresh metal complex with ammonium diethyldithiophosphate (DDPA) flows continuously around the micro-droplet, the analyte is extracted into the solvent. After that the drop is retracted back into the holding coil. A segment of $35 \mu\text{L}$ of the drop is propelled by an air stream and introduced into the graphite tube. The most important features of the system are: (i) facility of automated operation in micro-scale analysis, (ii) successful interfacing of the continuous operating process of SDME with discrete ETAAS and (iii) extremely low consumption of organic solvent thanks to the use of a syringe pump and an SIA manifold. The reliability of the proposed SI-SDME system was successfully demonstrated for cadmium determination in water samples.

In short, SDME is accessible to virtually all laboratories due to its simplicity, ease of implementation, and insignificant startup cost. Although some progress has been made to date to automate SDME, this technique is not yet

Table 1. Analytical performance characteristics of relevant flow-based LLE methods for atomic spectrometric determination of metals

Analyte	Flow method	LLE technique	Detection system	Linear range ($\mu\text{g L}^{-1}$)	Detection limit ($\mu\text{g L}^{-1}$)	Precision (%)	Enhancement Factor	Sample matrix	Ref.
Pb	SI	LLE	FAAS	3.0–250	1.4	2.9	120	Tap water, natural waters	42
Pb	FI	SPE-LLE	FAAS	NR	0.3	2.4	550	Tap, rain, and seawater	45
Pb	Hybrid FI/SI	LLE back-extraction	ICP-MS	0.02–0.5	0.017	4.8	23.3	Sea lettuce, river sediment, natural water, urine	38
Pb	FI	SLME	FAAS	170–1170	170	NR	10	River sediment (CRM 320)	77
Pb	FI	SLME	ETAAS/FAAS	5–80	0.1 (ETAAS) 6 (FAAS)	~ 4 (FAAS)	≤ 200	Urine	81
Pb	FI	CPE	ETAAS	0.2–4.0	0.045	2.9	22.5	Prawn, tea, rice, peach leaf	61
Pb	FI	CPE	ETAAS	0.2–35	0.016	5.1	21.6	Human hair, river water	62
Pb	CF	SDME	ETV-ICP-MS	0.01–50	8.3×10^{-4}	14	70	Human hair and urine	97
Pb	CF	SDME	ETV-ICP-MS	0.01–50	2.9×10^{-3}	12	190	Tap water, lake water, human serum, peach leaf	99
Cd	FI	Single-step LLE	FAAS	0.06–6.0	0.02	3.2	155	Natural water, seawater, river water, drinking water	17

Cd	FI	Single-step LLE	ETAAS	0.006–0.3	2.8×10^{-3}	3.2	24.6	Natural water, seawater, river water, drinking water, urine	39
Cd	SI	LLE back-extraction	ETAAS	0.05–0.8	2.7×10^{-3}	1.8	21.4	Sea lettuce, River sediment, natural water	18
Cd	FI	SLME	ETAAS	NR	0.1	≤ 6.8	7.7	Aqueous standards	79
Cd	FI	CPE	ICP-OES	0.5–100	0.1	2.3	71	River water, well water, and seawater	64
Cd	CF	SDME	ETV-ICP-MS	0.01–50	4.6×10^{-3}	16	140	Tap water, lake water, human serum, leaves	99
Cd	CF	SDME	ETV-ICP-MS	0.01–50	1.6×10^{-4}	12	300	Human hair and urine	97
Cd	SI	SDME	ETAAS	0.03–0.6	0.01	3.9	10	Tap water, river water, seawater	100
Cr(VI)	FI	Single-step LLE	ETAAS	0.005–0.6	3.3×10^{-3}	0.83	18.4	Aqueous NIST standard, seawater, waste water	46
Cr(VI)	Hybrid FI/SI	Single-step LLE	ETAAS	0.01–0.08	3.2×10^{-3}	0.80	18	Aqueous NIST standard	47
Cr(III)/Cr(VI)	FI	SLME	ETAAS	0–40	0.01 (Cr(VI))	≤ 9	24 (Cr(VI))	Surface water from tannery site	82
Cr	FI	CPE	ICP-OES	0.5–100	0.012 (Cr(III))	0.2	54 (Cr(III))	River water, well water, and seawater	64
Cu	Hybrid FI/SI	LLE back-extraction	ICP-MS	0.02–0.8	0.011	4.4	29.6	Sea lettuce, river sediment, natural water, urine	38

(Continued on next page)

Table 1. Analytical performance characteristics of relevant flow-based LLE methods for atomic spectrometric determination of metals
(Continued)

Analyte	Flow method	LLE technique	Detection system	Linear range ($\mu\text{g L}^{-1}$)	Detection limit ($\mu\text{g L}^{-1}$)	Precision (%)	Enhancement Factor	Sample matrix	Ref.
Cu	FI	CPE	ICP-OES	0.5–100	0.4	2.2	96	River water, well water, and seawater	64
Cu	FI	WFE	FAAS	≤ 100	0.2	1.5	43	Tap and river water	67
Cd	FI	WFE	FAAS	1.5–45	0.7	3.9	35	Tap water, river water, seawater, waste water	67
Cu	FI	SLME	ETAAS	NR	0.1	≤ 8.2	8.5	Aqueous standards	79
Co	CF	SDME	ETV-ICP-MS	0.01–50	5.6×10^{-4}	14	215	Human hair and urine	97
Co	FI	CPE	ICP-OES	0.5–100	0.3	4.0	97	River water, well water, and seawater	64
Sb(III)	FI	CPE	ETV-ICP-OES	NR	0.09	4.3	872	Tap water, natural waters, urine	63
Hg	FI	LLE	AFS	NR	0.02	5.6	5	Soil, geological samples, fly ash	44
Be	FI	Single-step LLE	ICP-OES	0.04–1000	8×10^{-3}	2.2	1300	Aqueous standards	40
Be	CF	SDME	ETV-ICP-MS	0.01–50	7.2×10^{-5}	16	260	Human hair and urine	97
Al	FI	SLME	ETAAS	NR	0.15	≤ 9.9	7.3	Aqueous standards	79
Al	CF	SDME	ETV-ICP-MS	0.5–100	3.3×10^{-3}	10	210	Natural waters, tap water, coffee and tea infusions	98
Fe(III)	SI	Single-step LLE	FAAS	100–6000	30	3.0	1.45	Wine	49
Fe	FI	CPE	ICP-OES	5.0–250	2.2	4.6	42	River water, well water, and seawater	64

Mn	FI	CPE	ICP-OES	0.5–100	0.1	3.0	60	River water, well water, and seawater	64
Bi	Hybrid FI/SI	Two stage LLE	ETAAS	0.05–2.0	0.0105	1.9	31.5	River sediment, urine	48
Gd	FI	CPE	ICP-OES	<50	0.04	1.9	20	Urine	59
Dy	FI	CPE	ICP-OES	<100	0.03	2.2	50	Urine	60
²³⁹ Pu/ ²⁴⁰ Pu	FI/SI	EChr	ICP-MS	NR	6.4 × 10 ⁻⁷ (²³⁹ Pu) 1.9 × 10 ⁻⁷ (²⁴⁰ Pu)	<3.4 (²³⁹ Pu) <5 (²⁴⁰ Pu)	<14.7 (EChr)	Seawater	87
²³⁹ Pu/ ²⁴⁰ Pu	FI	EChr	ICP-MS	NR	0.21 mBq L ⁻¹ (²³⁹ Pu) 0.19 mBq L ⁻¹ (²⁴⁰ Pu)	<4.6	2.2	Urine	89
²³⁹ Pu/ ²⁴⁰ Pu	FI	EChr	ICP-MS	NR	3 × 10 ⁻⁶ mg kg ⁻¹ (²³⁹ Pu) 3 × 10 ⁻⁷ mg kg ⁻¹ (²⁴⁰ Pu)	<2.0	2.5	Soil and sediment	91
²³⁸ U and ²³² Th	FI	EChr	ICP-MS	NR	2 × 10 ⁻⁵ (²³⁸ U) 3 × 10 ⁻⁵ (²³² Th)	2 (²³⁸ U) 3 (²³² Th)	4	Urine	90
²³⁹ Pu/ ²⁴² Pu	FI	EChr	ICP-MS	NR	2.9 × 10 ⁻⁶ mg kg ⁻¹ (²³⁹ Pu) 4.6 × 10 ⁻⁷ mg kg ⁻¹ (²⁴² Pu)	NR	2.5	Apple leaves	92

Acronyms: CF: Continuous flow, FI: Flow injection, SI: Sequential injection, LLE: Liquid-liquid extraction, CPE: Cloud-point extraction, WFE: Wetting-film extraction; SLME: Supported liquid membrane extraction, SDME: Single-drop microextraction, ETAAS: Electrothermal atomic absorption spectrometry, FAAS: Flame atomic absorption spectrometry, ICP-OES: Inductively coupled plasma optical emission spectrometry, ICP-MS: Inductively coupled plasma-mass spectrometry; ETV: Electrothermal vaporization; ETV: Electrothermal vaporization. NR: Not reported.

suitable as a routinely applicable on-line preconcentration procedure as a consequence of the complexity of manifolds. New materials for syringe needles that provide better adhesion to extractant solvent should be designed for improved analytical performance of the continuous-flow mode of SDME.

Where Is Flow-Based LLE Heading?

LLE should be regarded as one of the most progressive sample processing techniques for improvement of selectivity and sensitivity in many branches of analytical chemistry, including environmental and biological assays of trace elements. In addition, FI/SI-LLE methods are usually simpler, easier to handle, and more rapid and accurate than off-line batch counterparts. Actually, completely enclosed FI and SI setups prevent sample contamination and prevent the operator from coming into contact with potentially harmful solvents. However, the Achilles' heel of classical FI-LLE methods was the need for more effective and robust phase separators, which was overcome by the advent of novel LLE strategies, e.g., CPE, WFE, and EChr, as described in this review. Table 1 compiles the analytical performance of flow-based LLE systems as hyphenated to atomic spectrometric detection devices for trace-level determination of metal species.

The miniaturization of solvent extraction procedures leads inherently to a minimum solvent and reagent consumption and dramatic reduction of laboratory wastes. At this juncture, LOV systems offer vast potentials. It also might be a fruitful field for hyphenation with the very hot field of microfluidic systems. New perspectives are to be certainly opened in the near future for trace-metal monitoring by hyphenation of miniaturized LOV-LLE or LPME systems with ICP-OES/ICP-MS for multielemental determinations exploiting back-extraction protocols.

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