Anal Bioanal Chem (2006) 386: 739–748
DOI 10.1007/s00216-006-0467-5

ORIGINAL PAPER

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Highly selective micro-sequential injection lab-on-valve (μSI-LOV) method for the determination of ultra-trace concentrations of nickel in saline matrices using detection by electrothermal atomic absorption spectrometry

Received: 31 January 2006 / Revised: 24 March 2006 / Accepted: 4 April 2006 / Published online: 25 May 2006

Abstract A highly selective procedure is proposed for the determination of ultra-trace level concentrations of nickel in saline aqueous matrices exploiting a micro-sequential injection Lab-On-Valve (μSI-LOV) sample pretreatment protocol comprising bead injection separation/pre-concentration and detection by electrothermal atomic absorption spectrometry (ETAAS). Based on the dimethylglyoxime (DMG) reaction used for nickel analysis, the sample, as contained in a pH 9.0 buffer, is, after on-line merging with the chelating reagent, transported to a reaction coil attached to one of the external ports of the LOV to assure sufficient reaction time for the formation of $Ni(DMG)_2$ chelate. The non-ionic coordination compound is then collected in a renewable micro-column packed with a reversed-phase copolymeric sorbent [namely, poly(divinylbenzene-co-N-vinylpyrrolidone)] containing a balanced ratio of hydrophilic and lipophilic monomers. Following elution by a 50-μL methanol plug in an air-segmented modality, the nickel is finally quantified by ETAAS. Under the optimized conditions and for a sample volume of 1.8 mL, a retention efficiency of 70 % and an enrichment factor of 25 were obtained. The proposed methodology showed a high tolerance to the commonly encountered alkaline earth matrix elements in environmental waters, that is, calcium and magnesium, and was successfully applied for the determination of nickel in an NIST standard reference material (NIST 1640-Trace elements in natural water), household tap water of high hardness and local seawater. Satisfying recoveries were achieved for all spiked environmental water samples with maximum deviations of 6 %. The experimental results for the standard reference material were not statistically different to the certified value at a significance level of 0.05.

Keywords Brines · Nickel · Lipophilic/hydrophilic beads · Micro-sequential injection lab-on-valve · Preconcentration

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Introduction

Electrothermal atomic absorption spectrometry (ETAAS) is one of the most sensitive, well-developed and popular analytical techniques for the analysis of trace level concentrations of metal elements. However, their direct determination is still regarded to constitute a challenge to analytical chemists when it comes to the analysis of environmental water samples, such as seawater and brines,

which generally contain very low contents of the analyte ions (often sub-μg L^{-1} levels) and inherently high levels of alkaline and alkaline earth elements that can give rise to considerable spectroscopic and non-spectroscopic interferences in the detection step. In this context, a pretreatment procedure involving the separation of the targeted ions from the interfering matrix constituents, plus a concomitant preconcentration of the analyte species to fall within the dynamic operational range of the ETAAS instrument, is often a must.

Compared with the conventional batch mode, flow injection (FI) analysis [1–3] constitutes an attractive approach for automated solution manipulation including on-line sample pretreatment [4–7]. The second generation of FI, that is, sequential injection (SI) [8–10], offers merits such as automation, miniaturization, low consumption of sample and chemical reagents, and hence a minimal production of waste, and, being an enclosed system, low risks of contamination. In addition, it is regarded as a very promising tool for sample separation and preconcentration [11]. Further developments of SI involving the incorporation of a micro-conduit manifold mounted atop a selection valve, the so-called Lab-On-Valve (LOV), greatly extends the functions and versatility of the approach to allow a multitude of unit operations [12].

Solid phase extraction (SPE) [13] is the most attractive sample processing technique that can be readily miniaturized in the LOV unit. In fact, the application of the bead injection (BI) mode [14] in μSI-LOV for SPE has made it a very powerful micro-analytical tool in recent years [12, 15, 16].

Classified by their properties, the sorbent materials employed for μSI-BI-LOV fall into two categories: that is, the hydrophilic ones [17–20] and the hydrophobic ones [16, 21–23]. The most popular hydrophilic sorbents are ion-exchangers, including sulfopropyl-Sephadex and Sepharose beads. The main advantages of these materials are their commercial availability, trouble-free automatic handling within micro-conduit systems (e.g. LOV microchannels) and high retention efficiencies, thus implying high enrichment factors. In addition, they are applicable for a wide range of transition and heavy metals. Their disadvantage resides in the lack of selectivity due to the small differences in stability constants for the various metal ions and, even, between groups of elements. Hence, when such sorbent materials are employed for a sample containing large amounts of alkaline earth elements, their ability to retain a given trace metal becomes impaired by the lack of exchange capacity as a consequence of the saturation of the resin with the interfering ions. Therefore, functionalized sorbents with chelating moieties [13] or tailor-made ion-imprinted polymers (IIPs) [24] have been introduced for selective trace metal preconcentration. Even though the metal recognition capabilities of IIPs are well-established, the poor solubility of the analyte (template) in the imprinting mixture and the bleeding of unleached template with time limit their actual applicability [24]. In addition, due to difficulties associated with the synthesis of these materials, their commercial availability is rather scarce.

An appealing alternative to ion-exchange or ion-recognition processes is based on the exploitation of hydrophobic sorbents for the uptake of non-charged chelates generated by the reaction of the analyte with an appropriate, ideally selective, complexing reagent. The main benefit of this kind of sorptive material rests in the potentially high selectivity that can be obtained through an intelligent selection of the chelating reagent; that is, only the target ion, or preciously few other ions, are complexed and thus retained. Any positively or negatively charged matrix ion cannot be collected by the sorbent material. Even if some ions happen to be retained on the surface of the beads by weak interactions, they can be stripped out by the carrier solution itself during a cleansing step following sample loading. Another asset is that the chelate readily can be eluted by water-miscible organic solvents via a change in the polarity of the carrier solution rather than by employing strong acids to release the metal bound to the organic ligand. On-line LOV-based micro-fluidic procedures using this highly selective technique coupled to detection by atomic absorption or emission spectrometry have attracted extensive research interest in recent years [16, 21, 22]. However, a problem is to reconcile the disparity between the hydrophobic properties of the beads and the environment of the aqueous solution used, which sometimes makes the handling of the sorptive entities in the micro-channels of the LOV troublesome. Consequently, appropriate measures (e.g. bead re-circulation under continuous stirring and repetitive bead disposal protocols) must be taken to enhance the analytical performance of the bead-injection (sorbent renewable) approaches [22].

Precipitation might be regarded as an interesting option to SPE for on-line separation and preconcentration in flow systems [25]. Among the various techniques used for collecting the precipitate, knotted reactors (KR) [5, 7, 26, 27] feature low-flow resistance and high concentration factors, but at the expenses of low retention efficiencies (30–60 %). Membrane filters [28–30] have also been employed for precipitate collection, and despite their high efficiency they frequently give rise to the generation of flow impedance due to the accumulation of fine particles. Wang et al. [23] have recently reported that the use of a renewable micro-column in μSI-LOV, as employed to collect a cadmium hydroxide precipitate through surface adsorption, provided a promising approach to eliminating the above-mentioned drawbacks associated with the buildup of flow resistance and malfunctions of the sorbent surface.

It is well known that Ni(II) and dimethylglyoxime (DMG) share a very characteristic, selective reaction and that a neutral coordination compound is produced. It has been shown that octadecyl (C_{18})-chemically modified silica gel in a permanent packed column mode is a suitable medium for the collection of the $Ni(DMG)_2$ precipitate through hydrophobic interactions [31]. However, the use of the permanent column mode is often prone to problems due to the presence of other neutral organic compounds in the sample which can also be retained on the beads and give rise to some irreversible changes of the sorbent surface.

In the investigation reported here, we used a μSI-LOV system in the renewable bead-injection SPE mode to selectively determine Ni(II) by means of a lipophilic/hydrophilic copolymeric sorbent (poly(divinylbenzene-co-N-vinylpyrrolidone)) for the extraction and preconcentration of $Ni(DMG)_2$. The rationale for this choice is that the material, as opposed to conventional hydrophobic sorbents such as polytetrafluoroethylene and octadecyl-chemically modified silicagel [16, 22, 32], not only possesses a superior reversed-phase retention capacity but also entails a trouble-free handling in the μSI-LOV micro-conduits. Although the polymeric material is a commonly used SPE sorbent for the isolation of both neutral and acidic/basic organic pollutants prior to liquid or gas chromatography [33, 34], to the best of our knowledge its analytical application as a sorptive preconcentration medium for the isolation and preconcentration of metal chelates has not been reported to date.

Experimental

Instrumentation

The μSI-LOV-ETAAS system consisted, as depicted in Fig. 1, of the following components: a sequential injection system (FIAlab-3,000, Bellevue, Wash.), a home-made integrated LOV unit, an atomic absorption spectrometer (AAnalyst 600; Perkin Elmer, Foster City, Calif.) furnished with a longitudinal Zeeman background corrector and a transversely heated graphite furnace, equipped with pyrolytically coated graphite tubes, and an AS-800 autosampler. A wavelength of 232.0 nm with a spectral bandpass of 0.2 nm and an operating current of 25 mA were set for the nickel hollow cathode lamp (Perkin Elmer). The temperature program for the graphite atomizer is listed in Table 1. The signals were recorded in the integrated (peak area) mode.

The sequential injection system comprised a six-port selection valve (SV) atop of which was mounted the central sample processing unit named LOV. The LOV was connected to a holding coil (HC), a reaction coil (RC), high-precision bi-directional syringe pumps (SP1: 5 mL; SP2: 2.5 mL) and a peristaltic pump. The two-way valves at the heads of the syringe pumps facilitated the communication of each syringe either with an external container, which provided carrier or auxiliary reagent, or with the manifold. A PEEK T-connector was used for connecting SP2 to RC. The volumes of HC and RC were 3.0 mL and 2.5 mL, respectively, corresponding to 170 cm and 142 cm of 1.50-mm (i.d.)/2.10-mm (o.d.) polytetrafluoroethylene (PTFE) tubing, respectively. All other connecting tubes were made from 0.50-mm (i.d.)/1.66-mm (o.d.) PTFE tubing. The peristaltic pump, furnished with Tygon tubing of 1.22 mm (i.d.)/2.80 mm (o.d.), was utilized for filling the conduit from the external sample reservoir to port 5 with fresh sample solution prior to the analysis of the ensuing sample.

The dedicated LOV processing unit (diameter: 5 cm, thickness: 1 cm), which was made from hard polyvinyl chloride (PVC), contained a central port and six micro-channels [1.66 mm (i.d.)/12.0 mm (length)]. The communication between them was effected through a central micro-channel (CC) in the SV. PEEK stoppers (Upchurch Scientific, Oak Harbor, Wash.) were introduced in port 4 as well as in the central port connecting HC with CC in order to facilitate the formation of micro-columns C_1 and C_2. The stoppers, having a dimension slightly smaller than that of the channel, allowed the solution to flow freely while entrapping the beads in the respective cavities.

All of the operations of the μSI-LOV system were controlled by the associated FIAlab software (FIAlab

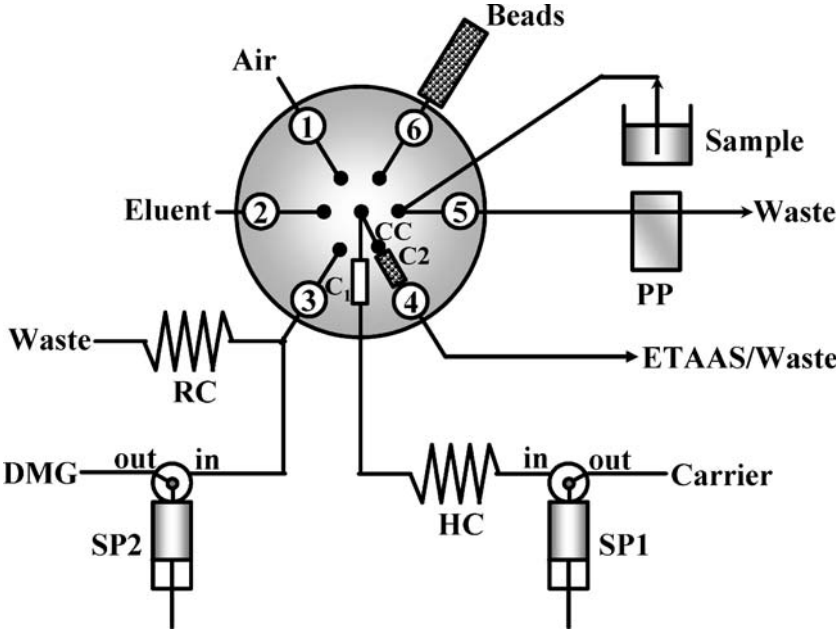

Fig. 1 Schematic diagram of the μSI-LOV-ETAAS system for on-line determination of Ni(II) via precipitation with DMG and preconcentration on poly (divinylbenzene-co-N-vinylpyrrolidone) beads. *Carrier* 0.2 mol L^{-1} ammonium citrate buffer (pH 9.0), *DMG* 1.2 % (w/v) dimethylglyoxime in ethanol, *eluent* methanol, *SP1* and *SP2* syringe pumps 1 and 2, C_1 and C_2 LOV micro-column positions, *HC* holding coil, *RC* reaction coil, *CC* central communication channel, *PP* peristaltic pump, *ETAAS* electrothermal atomic absorption spectrometer

Table 1 Temperature program for the ETAAS determination of nickel

Step	Temperature (°C)	Ramp time (s)	Holding time (s)	Argon flow rate (mL min^{-1})
Drying 1	100	5	20	250
Drying 2	130	5	20	250
Pyrolysis	1100	10	30	250
Atomization	2150	0	5	0
Cleansing	2500	1	4	250

Instruments, Bellevue, Wash.) and synchronized with the commands for the activation of the temperature program of the ETAAS instrument through an intelligent electronic interface [35]. The μSI-LOV system was actually programmed to execute the analytical protocol for the next analysis cycle while the former sample was pyrolyzed and atomized in the graphite furnace.

A digital pH meter (PHM92 LAB pH Meter; Radiometer A/S, Copenhagen, Denmark) was employed for measuring the pH of the solutions.

Reagents and solutions

All reagents were of analytical-grade, and doubly deionized water (18.2 MΩ cm), obtained from a Milli-Q Synthesis A10 system (Millipore, Molsheim, France), was used throughout.

An ammonium citrate stock solution with a concentration of 1.0 mol L^{-1} was prepared by dissolving 22.619 g of salt in 80 mL of water and adjusting the pH to 9.0 by the addition of concentrated ammonium hydroxide prior to the addition of water to a final volume of 100 mL. The carrier was obtained by diluting the 1.0 mol L^{-1} ammonium citrate buffer to 0.2 mol L^{-1} with water. Ni(II) working standard solutions were prepared from a 1000 mg L^{-1} Ni(II) stock solution (Merck, Darmstadt, Germany) by stepwise dilution with the 0.2 mol L^{-1} ammonium citrate buffer. A 1.2 % (w/v) DMG solution was prepared by dissolving 1.2 g DMG in 100 mL ethanol. Methanol was used as eluent.

Poly(divinylbenzene-co-N-vinylpyrrolidone) (Oasis HLB) beads with an average dry particle size of 30 μm and a specific surface area of 800 m^2 g^{-1} were purchased from Waters (Mildford, Mass.). Working suspensions were prepared by moistening 0.1 g of beads with 2 mL methanol. The analytical performance of these copolymeric beads was compared with that of an octadecyl chemically modified poly(styrene-divinylbenzene) material (C_{18}-PS/DVB) (Polysorb MP-1; Transgenomic, Omaha, Neb.) having a mean particle size of 40 μm. Practical slurries (1:20; w/v) were obtained by suspending the beads in 15 % (v/v) ethanol/water. The individual sorbent suspensions were aspirated into a plastic syringe (1.0 mL) which is mounted atop port 6 of the LOV unit. The tip of the syringe is furnished with a piece of PEEK tubing [0.76 mm (i.d.)/1.6 mm (o.d.)] and a conventional PTFE fitting with ferrule to allow for a tight connection to the LOV port.

The environmental water samples, including hard tap water and seawater, were filtered through a 0.45-μm pore size membrane filter, and then 10 mL of the 1.0 mol L^{-1} ammonium citrate pH 9.0 buffer was added to the 40-mL sample.

The standard reference material SRM 1640 (Natural water) was from the National Institute of Standards and Technology (NIST). As a consequence of the high content of nickel (namely, 27.4±0.8 μg L^{-1} Ni), the SRM was prediluted with the citrate buffer so that the final solution contained a concentration of 0.2 mol L^{-1} ammonium citrate buffer (pH 9.0).

Operating procedure

Before starting the analytical sequence, the tubing connecting the eluent reservoir (port 2) to the LOV, the line connecting SP2 to the T-connector and HC were filled with eluent (methanol), complexing reagent (1.2 % DMG) and carrier (0.2 mol L^{-1} ammonium citrate buffer, pH 9.0), respectively.

Table 2 summarizes the operations of the syringe pumps, the LOV positions, the volumes aspirated/dispensed and concomitant flow rates. Each analytical cycle includes seven steps, as described in the following:

Step 1:
System preconditioning. Firstly, both the line of the ETAAS auto-sampler arm and the RC are rinsed with 2000 μL of carrier from SP1. SP1 and SP2 are then loaded with 1750 μL of carrier and 100 μL of complexing reagent, respectively. Thereafter, the central channel is directed to port 1 for aspiration of 400 μL of air. Next, SP1 and SP2 are operated synchronously: SP2 is programmed to discard the first 40 μL of complexing reagent to waste (port 3) in order to minimize the DMG concentration gradient occurring at the tee-confluence, and SP1 is made to deliver 300 μL of air to RC via port 3 for the separation of the remaining solution in RC from the oncoming sample. The last 100 μL of air are left in the HC.

Step 2:
Sorbent preparation. A metered portion of beads is introduced from port 6 to C_1 and then the central channel is connected to port 2 for the aspiration of 400 μL of methanol. The remaining 100 μL of air in HC serve as a spacer between the carrier and methanol solutions to prevent the undesired overlapping of segments. The central channel is then directed to port 4, and a total volume of 1200 μL of solution, including methanol and carrier, is used for rinsing the sorbent material, which now becomes entrapped in micro-column C_2.

Step 3:
Sample pretreatment. SP1 is set to aspirate consecutively an air plug and 1800 μL of sample solution into HC. Before the mixing of sample and complexing reagent, 100 μL of sample is moved to the inlet of RC

in advance through port 3 to ensure that the sample and complexing reagent precisely merge at the mixing point of the T-connector. Afterwards, SP1 and SP2 move synchronously, thereby dispensing simultaneously 1800 μL of sample and 60 μL of complexing reagent into RC.

Step 4:

Analyte separation and preconcentration. With the central channel still directed to port 3, the interdispersed zones are pulled inwards into HC. The central channel then moves to port 4 to effect the collection of $Ni(DMG)_2$ onto the micro-column C_2. Afterwards, 500 μL of carrier are employed to rinse the analyte-loaded beads. A volume of 580 μL of air is next aspirated from port 1 into HC, from which 380 μL are used to direct the remaining solution in the ETAAS autosampler arm line to waste.

Step 5:

Activation of ETAAS. The ETAAS program is activated by a dedicated command in the SI program which allows the tip of the autosampler arm to move into the dose hole of the graphite tube for further delivery of eluate.

Step 6:

Elution. A minute, well-defined volume of methanol (50 μL) is aspirated from port 2. The central channel is then directed to port 4 and SP1 drives the eluent plug at a slow flow rate (10 μL s^{-1}) to pass through the

Table 2 Protocol for the determination of trace level concentrations of nickel by μSI-LOV-ETAAS

Step	Description	SP1 valve[a]	SP2 valve[a]	SP1	SP2	LOV position	Flow rate / μL s^{-1}	Volume /μL
1	System preconditioning							
	(a) Filling SP1 with carrier	Out		Aspirate			200	2000
	(b) Rinsing ETAAS line	In		Dispense		4	100	2000
	(c) Filling SP1 with carrier	Out		Aspirate			200	2000
	(d) Rinsing RC	In		Dispense		3	100	2000
	(e) Filling of SP1 and SP2 with carrier and complexing reagent, respectively	Out	Out	Aspirate	Aspirate		100 (SP1), 50 (SP2)	1750 (SP1), 100 (SP2)
	(f) Aspiration of air into HC	In		Aspirate		1	50	400
	(g) Introduction of air segment in RC and cleaning of the chelating reagent line	In	In	Dispense	Dispense	3	50 (SP1), 50 (SP2)	300 (SP1), 40 (SP2)
2	Sorbent preparation							
	(a) Beads loading	In		Aspirate		6	5	20
	(b) Methanol loading	In		Aspirate		2	50	400
	(c) Rinsing of beads and ETAAS line	In		Dispense		4	50	1200
3	Sample pretreatment							
	(a) Air segment introduction	In		Aspirate		1	50	100
	(b) Sample loading	In		Aspirate		5	100	1800
	(c) Filling the line between LOV and T-confluence with sample	In		Dispense		3	100	100
	(d) Merging sample with complexing reagent into RC	In	In	Dispense	Dispense	3	60 (SP1), 2 (SP2)	1800 (SP1), 60 (SP2)
4	Analyte separation and preconcentration							
	(a) Transportation of sample and reagent back to HC	In		Aspirate		3	50	1960
	(b) Delivery of interdispersed plugs to the LOV micro-column and rinsing of analyte-loaded beads	In		Dispense		4	20 (C_{18})/50 (copolymeric)	2460
	(c) Introduction of air into HC	In		Aspirate		1	50	580
	(d) Filling ETAAS line with air	In		Dispense		4	20	380
5	Activation of ETAAS							
6	Elution							
	(a) Aspiration of eluent	In		Aspirate		2	20	50
	(b) Dispensing of the eluent plug into column C_2	In		Dispense		4	10	50
	(c) Stopped flow for 5 s	In		–		4	–	–
	(d) Delivery of air-segmented eluate into the atomizer	In		Dispense		4	10	400
7	Bead discarding							
	(a) Dispensing carrier to C_2	In		Dispense		4	50	300
	(b) Bead transportation from C_2 to C_1 positions	In		Aspirate		4	50	300
	(c) Delivering of beads to waste	In		Dispense		3	50	300

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

analyte-loaded beads. SP1 is then halted for 5 s, whereupon the air-segmented eluate is transported into the graphite tube. Finally, the autosampler arm moves out of the furnace, and the ETAAS instrument continues to run the temperature program.

Step 7:

Bead discarding. The remaining 300 μL of carrier in the HC is dispensed to port 4 for subsequent backward aspiration of the used beads into HC, finally discarding them to the waste through port 3.

Results and discussion

Configuration of the μSI-LOV-ETAAS system

In this work, a hybrid FI-SI protocol is proposed for efficient radial mixing between the sample and complexing agent, thereby overcoming the limited reagent/sample overlapping inherent to conventional SI set-ups for large sample volumes [36]. Preliminary experiments were conducted using unidirectional forward-flow for simultaneous delivery of the sample (60 μL s^{-1}) and complexing reagent (3 μL s^{-1}) towards the beads contained in C_2. Thus, the T-confluence was located just before the micro-column position C_1. However, the analytical signals for 1 μg L^{-1} Ni were not significantly different from blank values. This was attributed to the short reaction time for on-line formation of the sparingly water-soluble chelate, which amounted merely to 2 s for the assembled flow configuration.

An alternative approach to facilitate the development of the reaction until completion is to increase the residence time for the interdispersed segments into the flow network. This can be elegantly performed in SI-manifolds via an auxiliary reaction coil that can be mounted to one of the peripheral ports of the selection valve. Hence, the mixture of sample and complexing reagent might remain there at will by the appropriate flow programming to acquire sufficient reaction time prior to the application of backward-flow for the collection of the generated precipitate onto the packed micro-column. Using the flow rates quoted in Table 2, the total residence time for the mixture, without resorting to stopped flow approaches, amounted to approximately 70 s. As a result, an 8.5-fold higher signal improvement was attained. To assess the dependence of the system's performance on the reaction time, a stopped-flow command was implemented in the SI program. No significant improvement in method's sensitivity was observed for residence times ranging from 70 to 250 s. Therefore, a reaction time of 70 s sufficed for the on-line generation of $Ni(DMG)_2$.

Different physical configurations and materials for RC were investigated. A knotted reactor (KR) provided the best mixing conditions, but, in the present case, gave rise to an impaired performance due to both the build up of back pressure during the flow-reversal aspiration stage and possible retention of the sparingly water-soluble chelate material on the tubing walls; consequently this option was discarded. Experimental results provided evidence for the nonexistence of significant differences in the analytical readouts at a confidence level of 95 % by exploiting either hydrophobic or hydrophilic tubing (namely, PTFE, Nylon and Tygon) as the open tubular reactor. The reason possibly lies in the fact that a coiled tubing with a large inner diameter (i.e. 1.5 mm i.d.) was employed, and the residence time of the mixture in the RC was relatively short, whereby the interactions of the $Ni(DMG)_2$ precipitate with the reactor were negligible.

The use of air-segmentation during the operational sequences was aimed at defining discrete solution segments in the flow network and at concomitantly preventing undesirable dispersion. Thus, the sample segment could be readily transported in a forward-backward fashion without dispersion into the carrier solution. During the preparation and washing of the beads, the air plug was employed to separate the methanol from the carrier in order to prevent the overlapping of solutions, which might cause the unwanted pre-elution of retained analyte during the clean-up step performed with a metered volume of carrier. An air-based sandwich strategy was also exploited in the elution step to meter a minute zone of eluate to satisfy the volume requirements of the graphite tube.

Parameter optimization

Chemical variables

When non-selective sorbents such as ion-exchangers or chelators are used for SPE for highly saline samples, several avenues have been proposed to improve the tolerance level to interfering matrix constituents. One approach relies on the implementation of a special cleansing step after sample loading that involves the removal of the loosely bound interfering ions by an electrolyte with a weak eluting strength, such as ammonium acetate, prior to the elution of the targeted species [37–39]. The main shortcoming is that it requires an additional operational step, and the analyte might subsequently be partially desorbed during the washing step. Masking reagents or pre-separation steps can be also applied for selectivity improvement. All of these measures are tedious and time-consuming, and sometimes the effect is not satisfactory due to the high level concentrations of potentially interfering elements (e.g. alkaline earth metals) in the sample matrix.

The benefit of using hydrophobic sorbents in SPE lies in the possible intelligent selection of the ligand for selective collection of the target species on the sorbent as a non-ionic complex, while all major matrix constituents remain in the liquid phase. Therefore, the adoption of a hydrophobic sorbent is a very promising approach for the analysis of highly saline samples.

Poly(divinylbenzene-co-N-vinylpyrrolidone) is a hydrophilic-lipophilic, water-wettable reversed-phase sorbent that was selected in this work for the purpose of scavenging

non-charged metal chelates via hydrophobic interactions. The commercially available sorbent has an average dry particle size of 30 μm, and the water-wettable beads can be readily captured in the LOV micro-column cavities. Under the microscope they are revealed to have perfectly spherical shapes and a uniform size distribution. In addition, this sorbent provides enhanced retention capacity for non-ionic and moderately polar species as compared with the traditional silica-based SPE sorbents like C_{18} [32, 40, 41].

With the exception of a few metal elements not commonly encountered in environmental waters, such as palladium, dimethyglyoxime (DMG) reacts selectively with nickel ions and forms a non-ionic colloidal precipitate. The mechanism of uptake of this coordination compound by the co-polymeric beads relies mainly on chemical interactions with the bead surfaces in lieu of physical filtration because hydrophilic sorbents, such as ion-exchange resins, render no appreciable collection of the target species. The complexation reaction involves the generation of oxonium ions, whereby the formation of the nickel chelate is favored under a buffered alkaline medium. Hence, the effect of pH on the yield of $Ni(DMG)_2$ formation was investigated, and the experimental results are shown in Fig. 2. As seen from this figure, the analytical performance improved at a pH>8, while pH 9.0 appears to be optimal; consequently, this latter value was chosen. An ammonium buffer was used for pH adjustment, thereby ensuring a high buffering capacity. However, a surplus of ammonia is inappropriate because the competitive complexation reaction of Ni(II) with ammonia might increase the solubility of $Ni(DMG)_2$. As a compromise between the above-mentioned factors, a buffer concentration of 0.2 mol L^{-1} was selected for the remainder of the studies.

To prevent the formation of insoluble oxyhydroxides of metal ions, such as Cr(III) and Fe(III), in real-life samples, which would in turn co-precipitate with the nickel chelate in the alkaline reaction medium and remain deposited on the LOV column, citrate was introduced to selectively form tightly bound soluble complexes with these metals. Ammonium citrate can thus not only serve as a buffer but also as a masking reagent for potential interfering species. Therefore, a 0.2 mol L^{-1} ammonium citrate buffer (pH 9.0) was employed for preparing the entire set of samples and working standards. The complexing reagent solution was prepared by dissolving DMG in absolute ethanol (1.2 %, w/v). To determine the optimal amount of chelating reagent for the on-line nickel precipitation reaction, various sample (1 μg L^{-1} Ni) to reagent (1.2 % DMG) ratios, ranging from 500 to 10, were assayed. It was found that the increase of DMG yielded improved absorbance signals until a ratio close to 20 was reached. Beyond this ratio, the method's sensitivity remained unchanged. DMG is only sparely soluble in water (0.063 g in 100 ml water at 25 °C), so the surplus of reagent might occasion its on-line crystallization into the flow conduits with the subsequent collection of particles onto the LOV packed micro-column, thus decreasing the retention efficiency for the target analyte. In this context, it should also be borne in mind that the Ni $(DMG)_2$ complex is highly soluble in ethanol. Thus, when the ratio of aqueous solution to reagent is affixed to 20, the ethanol content in the flow system is merely 5 % (v/v), while at a ratio of 10 it will be increased to 10 %. To prevent the partial dissolution of the adsorbed $Ni(DMG)_2$ by the sample medium itself, a minute volume of complexing reagent is therefore preferable. For this reason, a mixing ratio of 20 for sample to complexing reagent was employed for further investigations.

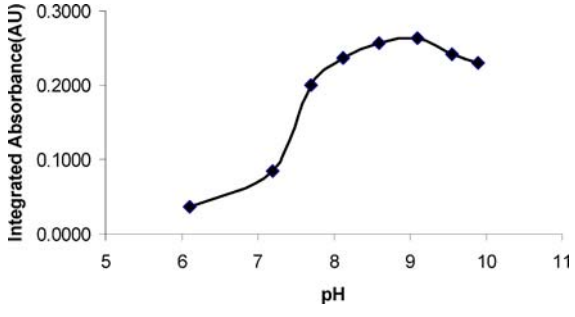

Fig. 2 Influence of sample acidity on the analytical signal recorded. Standard, 1 μg L^{-1}Ni(II); loading volume, 1.8 mL; sample to chelating reagent ratio, 20; loading flow rate, 50 μL s^{-1}, eluent volume, 50 μL

μSI-LOV variables and eluting procedure

To obtain the best performance in the separation and preconcentration of Ni(II) in the LOV unit, the ideal manifold should effectively retain the target species (i.e. the chelate) on the micro-column while permitting the stripping of the analyte quantitatively before its transportation to the detector. To this end, the SI parameters involving loading and elution of the micro-column were investigated.

The flow rates for the uptake of the $Ni(DMG)_2$ precipitate were varied from 10 to 100 μL s^{-1}. The highest integrated absorbances were obtained at flow rates <50 μL s^{-1}, while they decreased by 8 % at a flow rate of 70 μL s^{-1} and by 12 % at 100 μL s^{-1}. Thus, the sample loading flow rate was affixed at 50 μL s^{-1} for further experiments.

A very efficient approach to strip the target metal ion from the collection medium is by breaking the hydrophobic interaction between the solid-phase and the chelate, which can be effected by employing an organic reagent, such as methanol or ethanol. Even if some hydrolyzed metals should remain accumulated on the sorbent after the elution procedure, the analytical performance, as opposed to SPE in permanent columns [31], is not deteriorated with respect to repeatability because the bead injection approach entails the discarding of the used beads and the automatic column re-packing with a fresh portion of sorptive entities for each cycle.

It should be noted that the higher the eluent flow rate, the shorter the contact time between the stripping agent and the sorbent. Furthermore, flow rates >10 μL s^{-1} gave rise to multi-segmented zones, which might cause the incomplete delivery of the air-segmented eluate to the graphite tube. Therefore, a flow rate of 10 μL s^{-1} was finally adopted. A

stopped-flow mode, that is, the eluent is halted for a preselected time interval and remains within the analyte-loaded sorbent column to assure quantitative elution, was also investigated. The experimental results evidenced an improvement in the absorbance signals up to 10 % when the forward-flow elution was replaced by a stopped-flow procedure involving a halting time of 5 s.

An eluent volume of more than 50 μL is rarely allowed to be introduced into the ETAAS atomizer due to the physical dimensions of the graphite tube. In the present application, however, a 50-μL methanol plug was imperative for effective stripping of the sorbed compound since the analytical sensitivity dropped by 20 % whenever the elution was performed with 40 μL of methanol.

To assess whether or not carryover effects were negligible, a further elution step was conducted using an identical eluting protocol prior to sorbent renewal. Sample cross-contamination was calculated to be lower than 2.5 % under the experimental conditions selected, thereby proving that a single 50-μL eluent segment sufficed for effective stripping of the nickel chelate from the sorbent material.

Interferences

The tolerance of the μSI-BI-LOV method to foreign species was evaluated by using a fixed concentration of Ni(II) (0.8 μg L^{-1}) and various amounts of potentially interfering ions. The effect of the most commonly encountered ions in environmental waters – Ca(II), Mg(II), Pb(II), Cu(II), Cd(II), Fe(III), Mn(II) and Co(II) – was thoroughly investigated. The experimental results showed that the interference level was <10 % when the ratio of interfering species, such as Ca(II) and Mg(II), to analyte was below 1×10^6, while a ratio of interferent to analyte below 1×10^4 for Pb(II), Cu(II), Cd(II), Fe(III), Mn(II) and Co(II) caused no significant deviations in Ni(II) recoveries. The high tolerance to alkaline earth elements implies the possibility of direct analysis of hard waters and seawater, with no need for any sample pre-treatment (see below). The tolerance level to interfering ions was compared with another μSI-LOV method in which the ion-exchanger SP Sephadex C-25 was employed for the separation and preconcentration of Ni(II) in environmental and biological samples [42]. In addition to allowing a fivefold higher content of alkaline earth elements, the present method showed a tenfold higher tolerance to Co(II), Mn(II) and Cd(II).

Analytical performance of the μSI-LOV-ETAAS protocol with co-polymeric sorbent beads for the assay of nickel and its comparison with the use of C_{18}-PS/DVB

Under the optimized chemical and μSI-LOV operational parameters, the merit points of the bead-injection protocol exploiting poly(divinylbenzene-co-N-vinylpyrrolidone) as sorptive medium for the determination of nickel are summarized in Table 3, including the regression equation, linear dynamic range, required amounts of sorbent, sample and eluent, sampling throughput, enrichment factor and statistical parameters. The retention efficiency is defined as the ratio of the retained amount of nickel to the total amount of analyte available in the sample. The enrichment factor is determined as the ratio between the linear range sensitivity of the proposed method and that obtained by direct injection of 50 μL of a series of standards. The detection limit is defined as the concentration of analyte corresponding to a signal equal to threefold that of the standard deviation of the blank. Repeatability and reproducibility were obtained by seven consecutive measurements of a 0.8 μg L^{-1} Ni(II) standard using the permanent and renewable column fashion, respectively.

The performance of the poly(divinylbenzene-co-N-vinylpyrrolidone) copolymeric sorbent was compared with that of another reversed phase sorbent, namely, C_{18}-

Table 3 Analytical performance of the μSI-BI-LOV system exploiting N-vinylpyrrolidone-divinylbenzene beads for the determination of Ni(II) and its comparison with C_{18}-PS/DVB as a sorptive material

Parameter	Sorbent	
	C_{18}-PS/DVB	Poly (divinylbenzene-co-N-vinylpyrrolidone)
Regression equation	0.1969 (Ni, μg L^{-1}) +0.0698	0.2057 (Ni, μg L^{-1}) +0.0592
Correlation coefficient	0.9995	0.9978
Linear range/μg L^{-1}	0.2–2	0.2–2
Sorbent volume/μL	20	20
Sample volume/mL	1.8	1.8
Eluent volume/μL	50	50
Loading flow rate/μL s^{-1}	20	50
Sample frequency/h^{-1}	8	10
Retention efficiency (%)	66	69
Enrichment factor	24	25
Detection limit/μg L^{-1} (3σ)	0.04	0.05
Repeatability (%)	4.5	4.8
Reproducibility (%)	7.2	5.6

Table 4 Determination of trace level concentrations of Ni(II) in environmental waters by hyphenation of μSI-BI-LOV with ETAAS

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

[a]The results are given as the mean of three replicates ± standard deviation
[b]Dilution factor, 40:50

PS/DVB; both showed a very similar performance in most of the parameters listed except for the sample loading flow rate and statistical parameters. Thus, the C_{18}-PS/DVB material needs a relatively lower sample loading flow rate (20 μL s^{-1}) than the co-polymeric material (50 μL s^{-1}) to assure a complete collection of the nickel precipitate. In addition, C_{18}-PS/DVB has a poorer reproducibility. All of these factors can possibly be attributed to the difference in the hydrophobic/hydrophilic nature of the two sorbents. Since all of the micro-fluidic operations are conducted under an aqueous environment, the sorbent with more hydrophilic properties should benefit from this in performance. The hydrophilic monomers of the co-polymeric material can greatly facilitate the interaction between the surface of the beads and the aqueous solution, thereby allowing a faster mass transport on the liquid-solid interface. The reason for the better reproducibility of the water-wettable sorbent should be attributed to the straightforwardness of its handling in the micro-LOV channel system as compared with its counterpart.

Validation

Environmental water samples of relative matrix complexity, including hard tap water and seawater, as well as a certified reference material (SRM 1640) were employed to validate the reliability and accuracy of the proposed method using poly(divinylbenzene-co-N-vinylpyrrolidone) as a renewable sorbent for the separation and preconcentration of ultra-trace levels of nickel. The recoveries obtained for the environmental waters spiked at two different levels of Ni(II) are listed in Table 4. As can be seen from the table, maximum deviations of 6 % were found, thus indicating the absence of multiplicative (non-spectroscopic) matrix interferences. It should be taken into account that direct analysis of the local tap water (Kgs. Lyngby, Denmark), the source of which is an underground reservoir, using μSI-BI-LOV with ion-exchangers or chelating resins was previously proven to be cumbersome for trace element determination [43] owing to the high content of calcium – namely, 128 mg L^{-1} – which corresponds to a hardness of 18 °.

For SRM 1640, a concentration of 30.0±1.2 μg L^{-1} of Ni(II) was determined, which is in good agreement with the certified value (27.4±0.8 μg L^{-1}). A statistical *t*-test was performed to compare the means of the experimental result with the certified reference value [44]. No significant differences were found to be present at a confidence level of 95 %.

Conclusion

A lipophilic/hydrophilic copolymeric sorbent [poly(divinylbenzene-co-N-vinylpyrrolidone)] has been proposed, for the first time, as a sorptive material for selective trace element separation and preconcentration in complex environmental samples (hard waters and brines) utilizing a miniaturized and automated sequential injection LOV system in a bead-injection mode. Hyphenated with detection by ETAAS, the developed flow assembly has proven to be suitable for monitoring trace concentrations of Ni following on-line derivatization with DMG and collection of the organic chelate on the bead surfaces. Compared with hydrophilic sorbents, such as chelating Sepharose and Sephadex ion-exchangers, which have been used as renewable entities in the LOV unit, the co-polymeric material features an improved selectivity in the presence of alkaline earth metals as a consequence of its hydrophobic nature. Thus, in our particular case, ratios of Ca(II) or Mg (II) to Ni(II) as high as 1×10^6 might be tolerated. Furthermore, as opposed to conventional hydrophobic sorbents (e.g. polytetrafluoroethylene and octadecyl chemically modified silicagel), the polymeric beads entail a trouble-free automatic handling in the SI-LOV microconduits due to its hydrophilic/lipophilic balance.

The implementation of new materials as a preconcentration medium in LOV may thus expand the scope of applicability of the third generation of FI to biological and environmental matrices of increased complexity with no need for any ancillary off-line sample pre-treatment procedure.

Acknowledgements Xiangbao Long is grateful for a 3-year Ph.D. stipend granted to him by the Technical University of Denmark. Manuel Miró is indebted to the Spanish Ministry of Education and Science for financial support through the "Ramon y Cajal" research program.

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