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Talanta 66 (2005) 1326–1332
Talanta
www.elsevier.com/locate/talanta

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

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Received 9 December 2004; received in revised form 19 January 2005; accepted 26 January 2005
Available online 2 March 2005

Abstract

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

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

1. Introduction

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

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

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doi:10.1016/j.talanta.2005.01.056

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

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

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

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

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

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

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

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

2. Experimental

2.1. Instrumentation

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

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

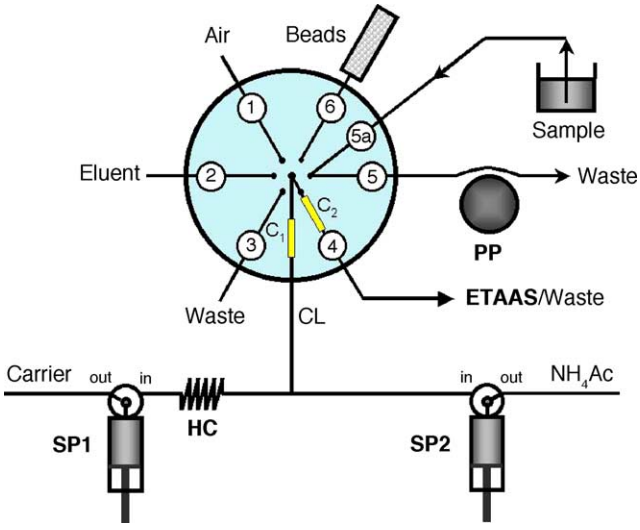

Fig. 1. SI-LOV system for determination of ultra-trace levels of Cd, Pb and Ni using preconcentration by chelating Sepharose beads and detection by ETAAS. SP1 and SP2, syringe pumps; PP, peristaltic pump; CL, communication line; C_1 and C_2, microcolumn positions (although these for clarity are shown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

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

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

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

2.2. Reagents

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

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

2.3. Sample pretreatment

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

2.4. Operating procedure

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

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

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

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

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

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

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

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

3. Results and discussion

3.1. Optimization of ETAAS parameters

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

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

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

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

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

3.2. Optimization of sample acidity

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

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

3.3. Optimization of SI-LOV parameters

3.3.1. Column cleansing

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

3.3.2. Effects of sample flow rate

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

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

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

3.3.3. Choice of eluent

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

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

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

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

3.3.4. Measurement of elements

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

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

3.4. Investigation of interferences

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

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

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

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

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

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

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

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

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

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

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

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

4. Conclusion

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

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

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

Acknowledgements

Xiangbao Long is grateful to the Technical University of Denmark (DTU) for allocating a Ph.D. stipend. Manuel Miró is indebted to the Spanish Ministry of Education and Science for financial support through the “Ramon y Cajal” Research Program. The authors wish to extend their appreciation to the mechanical workshop of the Department of Chemistry, headed by John Madsen, for invaluable help in designing and making the LOV units.

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