Integrated FIA/HPLC Method for Preconcentration and Determination of Transition Metal Ions

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Key Words

Summary

An automatic method based on the combination of a flow injection (FI) manifold with a liquid chromatograph has been developed for the enrichment and determination of transition metal ions in water samples. Alternatively, the FI configuration can be used as a screening system for the determination of the total concentration of heavy metals. Two-parameter expressions for calibration graphs involving preconcentration time and concentration of the analytes were established for both the FIA and the integrated FIA/HPLC methods. The preconcentration time depends on the concentration level of the analytes in the samples. The method is linear in the range 2–200 ng/ml, with r.s.d. values between 1.5 and 5.0. It has been applied to the determination of copper, lead, zinc, nickel, cobalt, cadmium and manganese in synthetic water samples.

Introduction

The coupling of Flow Injection Analysis and HPLC has been shown to provide very promising results as a screening system for the total and individual determination of groups of analytes having similar nature and chemical behaviour [1–3]. In addition, this approach offers a useful means for enhancing sensitivity in continuous photometric detection by post-column derivatization reaction [4–6].

The use of HPLC for separation and determination of inorganic ions has been recently reviewed by Robards et al [7]. Their work is aimed at the determination of metals by liquid chromatography with particular emphasis on the role of complexation. Several approaches for the determination of metals by chromatography have been described which differ in the mechanism of the separation process. One of these approaches is ion-chromatography (IC) which offers excellent results for simultaneous separations and determinations of transition metal ions in complex mixtures [8], particularly when ion interaction chromatography in conjunction with post-column derivatization is used.

In order to improved the sensitivity and selectivity of the trace analysis of metals, a preconcentration step prior to analysis has found to be very useful. Preconcentration processess offers both concentration of the analytes of interest from a large volume of sample and minimization of interferences from different species present in the samples. In this context, Terada [9] has critically reviewed the preparation and characterization of complex-forming adsorbents for the sorption of heavy metals, showing the advantages and disadvantages of each adsorbent.

Despite the fact that on-column preconcentration is capable of very high enrichment factors [10], this technique is not used to preconcentrate samples of high ionic strength since the total exchange capacity of this technique is usually very small [11]. Previous studies [12–13] have demonstrated that preconcentration on Chelex-100 is efficient any yields low blank signals and Olsen et al [14] developed an on-line flow injection preconcentration method using a Chelex-100 microcolumn for the preconcentration of lead. This permitted determination of Pb at concentrations as low as 10 ppb and cadmium and zinc at 1 ppb with no matrix interference from samples with a high saline content (sea water).

The aim of this work was to develop a simple, reliable and inexpensive preconcentration and derivatization system by using an FI manifold which can be used on its own for screening purposes and also as a precolumn concentration and post-column derivatization system for HPLC.

Experimental

Reagents

All chemicals used were analytical reagent grade. Deionized water (Millipore Milli-Q-System) was used throughout.

Standard solutions of the cation analytes were prepared by dilution of aqueous 1000 mg/l stock solutions.

The reagent for derivatization of transition metals was a solution of PAR; [4–(2-pyridylazo)-resorcinol monosodium salt] in ammonia-ammonium acetate buffer.

An iminodiacetic acid chelating resin Chelex-100 mesh (Sigma) was used for preconcentration and 0.1 M nitric acid as eluting solution. Before its use, the resin was converted to the ammonium form by washing with 0.05 M ammonium acetate solution [14].

The chromatographic column was an Ultrabase C-18 (4.6 × 25 mm; 5.0 μm) Sharlau Science). A solution containing 50 mM tartaric acid and 2 mM octanesulphonate sodium salt (OSS) (Aldrich Chemical Co.), pH, 3.5 was used as mobile phase.

Apparatus and Instruments

An HP 1050 system (high pressure quaternary gradient pump, Rheodyne 7125 manual injection system), an HP 40A diode array spectrophotometer equipped with an HP 1040 DAD flow cell (2 μl inner volume) coupled to an HP 9000 Chem Station were used. Two four-channel Gilson minipuls-2-peristaltic pumps furnished with a rate selector, two Rheodyne 5041 low pressure injection valves, one Rheodyne 5060 rotary valve, a 50 mm length microbore glass column and two Tecator TM II chemifolds were also used.

Manifold and Procedure

Figure 1 shows the FIA/HPLC integrated manifold system used. The FIA sub-system contained two injection valves in series. A Chelex-100 microcolumn was located in the loop of the second valve (IV2) in which samples of all metals studied were preconcentrated by passing the sample solution through the loop for a preset interval
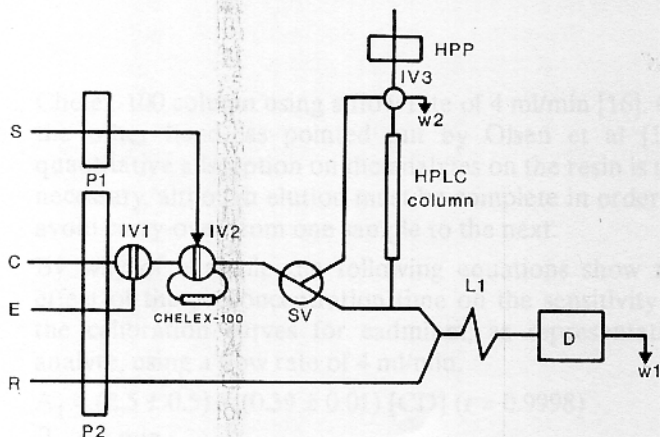

Figure 1

FIA/HPLC integrated manifold. S denotes sample, C, carrier (H_2O), E, eluting agent (0.1 M HNO_3), R, derivatizing solution (0.1 mM PAR in 0.4 M NH_3/0.2 M NH_4Ac), P low pressure pump, HPP, high pressure pump, IV1, IV2 and IV3, injection valves, Chelex-100 minicolumn of the dimensions given in Table I packed with resin, SV, the selecting valve, W, waste and D detector.

(Tp). The loop of the first injection valve (IV1) was filled with 0.1 M nitric acid. After the preconcentration time, which depends on the concentration of the analytes in the samples, valves IV2 and IV1 were sequentially switched in that order with an interval of 10 s. The nitric acid solution passed through the microcolumn and the concentrated metals were eluted quantitatively. When the selecting valve (SV) was in position 1, the metal ions in the sample reacted directly with PAR in L1 and the detector monitored the total concentration of metals present in the sample. Conversely, when SV was in position 2, the eluate was trapped in the loop of the HPLC valve (IV3) and injected into the chromatographic column. Each analyte then reacted separately with PAR in L1 after elution from the column.

Results and Discussion

The flow injection sub-system (Figure 1) can be used not only as a post-column reactor-detector but also as a preconcentration assembly for the enrichment of transition metals from water samples. On the other hand, the FI manifold can also be used as an introduction system to the HPLC where the individual analytes are isolated or as a screening system for determining the total concentration of heavy metals in the sample. After this screening, only those samples with an abnormally high concentration are injected into the liquid chromatograph where the concentration of each analyte ion is determined separately.

Optimization of Variables of the FIA Method

Flow injection variables were optimized by the univariate method and the optimum values found, shown in Table I, were then used to establish the method. The most influential chemical variables for the analytical signal and background noise were the concentration of PAR and that of the ammonia buffer. Although a 0.2 mM solution of PAR in 2 M NH_3/1 M NH_4Ac has been recommended [18], a 0.1 mM solution of PAR in 0.4M NH_3/0.2M NH_4Ac was found to yield a more stable baseline and also to be more stable to oxidation, thus avoiding the need to keep it under inert atmosphere.

Preconcentration Using a Chelex-100 Microcolumn

Taking into account suggestions made by other users [14–16], a Chelex-100 microcolumn was used to preconcentrate and separate transition metal ions from very dilute aqueous samples. This resin, in which the imino-diacetic acid moiety is incorporated into a styrene-DVB copolymer, has been used previously for selective enrichment of these analytes from aqueous solutions. Pai et al. [15] observed that low recoveries were obtained in sea water samples owing to the high concentration of calcium and magnesium present. These authors also suggested that, in order to achieve better performance, aqueous samples should be adjusted to pH 6.5 before loading onto a

Table I Study of variables

Variable[1]	Range studied	Optimum value
FIA subsystem		
Flow rate (P1), ml/min	1.1–4.5	3.2
Flow rate (P2), ml/min	0.9–4.2	4.0
Injected volume (IV1), µl	30–500	50
Length of reactor (L_1), cm	30–200	30
Length of CHELEX-100 column, cm	0.5–3.0	1.0 (50 mg)
Preconcentration time (Tp), cm	–	relative
Switching time[2], s	5–15	10
[HNO_3], mol/l	0.001–1.0	0.1
[PAR], mmol/l	0.01–2.0	0.1
[NH_3], mol/l	0.01–2.0	0.4
[NH_4Ac], mol/l	0.01–2.0	0.2
HPLC subsystem		
Flow rate (HPP), ml/min	0.3–1.5	1.3
Injected volume (IV3), µl	20–200	100
Switching time[3], s	30–40	35
[Tartaric acid], mmol/l	–	50
[OSS], mmol/l	–	2.0
pH	3.0–4.0	3.5

(1) Abbreviation as stated under experimental, (2) between IV2 and IV1 valves,
(3) between IV1 and IV3

Table II Features of the FIA method

Metal	Equation[1]	Correlation Coefficient	Detection limit[2] (ng/ml)	R.S.D %[3] (n = 11)
Cu	$A = -3.5 \pm 0.5 + (0.416 \pm 0.009)$ [Cu] Tp	0.9998	0.5	1.20
Pb	$A = 3.5 \pm 0.7 + (0.082 \pm 0.007)$ [Pb] Tp	0.9991	2.7	2.36
Zn	$A = -2.4 \pm 0.2 + (0.810 \pm 0.004)$ [Zn] Tp	0.9998	0.3	1.08
Ni	$A = 3.0 \pm 0.3 + (0.535 \pm 0.008)$ [Ni] Tp	0.9997	0.4	0.98
Co	$A = 3.3 \pm 0.7 + (0.682 \pm 0.001)$ [Co] Tp	0.9998	0.3	1.49
Cd	$A = 2.3 \pm 0.4 + (0.383 \pm 0.006)$ [Cd] Tp	0.9993	0.6	1.95
Mn	$A = -2.4 \pm 0.4 + (1.021 \pm 0.008)$ [Mn] Tp	0.9997	0.2	1.01

(1) A in miliabsorbance units, [ ], concentration in ng/mL, Tp, preconcentration interval (2) Tp = 5 min,
(3) 30 ng/ml of each metal, Tp = 5 min

Chelex-100 column using a flow rate of 4 ml/min [16]. On the other hand, as pointed put by Olsen et al [14], quantitative adsorption on the analytes on the resin is not necessary, although elution must be complete in order to avoid carry-over from one sample to the next.

By way of example the following equations show the effect of the preconcentration time on the sensitivity of the calibration curves for cadmium, as representative analyte, using a flow rate of 4 ml/min.

$A_1 = (2.5 \pm 0.5) + (0.39 \pm 0.01)$ [CD] (r = 0.9998)

$T_p = 1$ min

$A_3 = (5.9 \pm 0.7) + (1.19 \pm 0.01)$ [CD] (r = 0.9999)

$T_p = 3$ min

$A_5 = (10.1 \pm 0.9) + (1.91 \pm 0.02)$ [CD] (r = 0.9998)

$T_5 = 5$ min

As can be seen by the slopes of the calibration lines, the enrichment factor increases proportionally with the pre-concentration time. From these data, it was possible to establish the following general expression for calibration:

$$A = a [M]Tp + b$$

where A is the analytical response (FIA peak height) in miliabsorbance units, and a and b are the slope and intercept of the linear graph, respectively; [M] is the concentration in ng/ml and Tp is the preconcentration time in s. The linear equations established for each analyte as a function of the preconcentration time are shown in Table II. From the equations it is clear that transition metals can easily be determined at ppb level at preconcentration times shorter than five min. The precon-centration time, required to preconcentrate a measurable amount of an analyte, depends on its concentration level in the samples, so, theoretically, the detection limit of the method should be only limited by sample contamination. However, the 3σ detection limits were in the range 0.21–2.6 ng/ml for a preconcentration time of five min (Table

II). The precision of the method was tested with solutions of 30 ppb for each metal and the relative standard deviation values, also shown in Table II, were quite acceptable in all instances.

Optimization of the Integrated FIA/HPLC System

Among the different HPLC approaches reported for the determination of heavy metals, dynamic coating ion-interaction chromatography, which can be easily integrated with an FI system and usually preferred for cation separations [7], was selected for our FIA/HPLC arrangement.

The mobile phase selected was similar in composition to that reported earlier [17], and consisted of an aqueous solution of 50 mM tartaric acid and 2 mM sodium octane sulphonate. The solution was adjusted to pH 3.5 in order to obtain enough ionized tartaric acid to complex the analytes in the elution process.

After the metal ions were preconcentrated and eluted from the Chelex-100 microcolumn in the FIA system, they were quantitatively transferred into the HPLC column to carry out the separation step. For this purpose, a large volume HPLC injection valve (IV3 in Figure 1) was necessary to avoid lost of the analytes. The optimum analytical signals were obtained with a loop of 100 μl. In addition, the optimum time interval between injection of nitric acid as eluent of the preconcentrated metals and switching of the HPLC injection valve to insert the plug of the concentrated analytes into the chromatograph was found to be 35 ± 2 s.

The flow-rate ratio (q_{HPLC}/q_{FIA}) was studied over the range 0.09 to 0.47. The optimum ratio was 0.40 for a flow-rate of the FI manifold (q_{FIA}) of 3.20 m/min. Lower ratios resulted in smaller signals while higher ratios gave a too high overpressure.

Under these experimental conditions the separation of seven cations was complete in less than 15 min (Figure 2). The reaction with PAR occurs instantaneously and produce the well-known highly coloured complexes with maximal absorption at about 500 nm. A wavelength of 520 nm was selected for monitoring as a compromise between sensitivity and baseline noise. Peak-area meas-
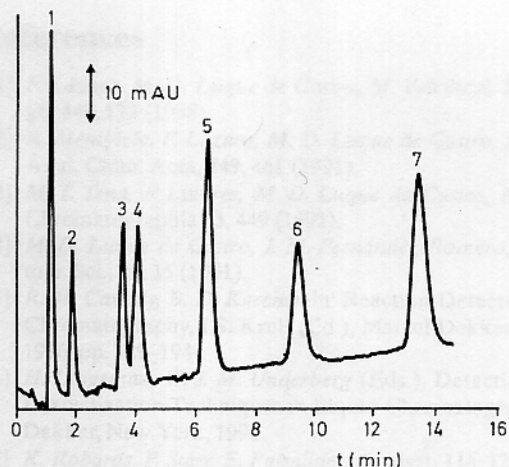

Figure 2

Separation of transition metal ions by HPLC/FIA integrated system. (1) 25 ng/ml Cu; (2) 70 ng/ml Pb; (3) 10 ng/ml Zn; (4) 15 ng/ml Ni; (5) 25 ng/ml Co; (6) 25 ng/ml Cd; (7) 15 ng/ml Mn. Chromatographic conditions: Column, Ultrabase C-18 (25 · 4.6 mm 5.0 mm); Mobile phase, 50 mM tartaric acid and 2mM OSS, pH 3.5; Flow rate, 1.3 ml/min. FIA conditions: Flow rate, (P1), 3.2 ml/min, (P2), 4.0 ml/min; Tp, 5 min; Preconcentration column, Chelex-100.

urements were performed rather than peak-height as a better reproducibility was achieved with area.

Table III shows the features of the integrated method. Calibration graphs were obtained separately for each metal ion and it was also possible to establish equations containing both concentrations of the analytes and preconcentration times. The reproducibility of seven separations of transition metals (Table III) reflects the reproducibility of the combined preconcentration-elution/chromatographic separation/post-column reaction-detection process. The detection limits shown in Table III were calculated taking into account the standard deviations of peak areas of solutions containing 30 ppb of each cation with a preconcentration time of 5 min.

The method was applied to the determination of seven transition metals in eight synthetic samples of water (containing 3 % w/v of NaCl) using the standard addition technique. Different samples were prepared at concentration levels ranging between 5 to 10 ppb. The results were found to be satisfactory as can be seen in Table IV for one representative sample.

Table III Features of the integrated method

Metal	Equation[1]	Correlation Coefficient	Detection limit[2] (ng/ml)	R.S.D %[3] (n = 11)
Cu	A = 12.47 + 1.76 [Cu] Tp	0.9992	2.5	2.52
Pb	A = − 1.94 + 0.23 [Pb] Tp	0.9995	24.4	4.54
Zn	A = − 8.19 + 4.23 [Zn] Tp	0.9997	4.2	5.09
Ni	A = −2.83 + 2.68 [Ni] Tp	0.9992	2.0	2.36
Co	A = 6.72 + 2.48 [Co] Tp	0.9993	6.6	4.05
Cd	A = − 2,38 + 1.39 [Cd] Tp	0.9996	2.3	1.56
Mn	A = − 6.20 + 4.93 [Mn] Tp	0.9999	4.9	1.59

[1]A in area units, [ ], concentration in ng/ml, Tp, preconcentration interva. [2]Tp = 5 min, [3]30 ng/ml of each metal, Tp = 5 min

Table IV Determination of transition metal ions by FIA/HPLC integrated system in a synthetic sample[1]

Metal	Concentration Added (ng/ml)	Concentration Found[2] (ng/ml)	Recovery %
Cu	20	20.95	104.7
Pb	40	39.00	97.5
Zn	5	5.27	105.4
Ni	20	21.03	105.1
Co	20	21.71	108.5
Cd	30	29.12	97.1
Mn	20	19.51	97.6

[1]A in area units, [ ], concentration in ng/ml, Tp, preconcentration interva. [2]Tp = 5 min, [3]30 ng/ml of each metal, Tp = 5 min

Conclusions

The integrated system proposed in this work is useful for the enrichment of trace levels of transition metal ions prior to FIA or HPLC determination. These analytes can be easily determined at ppb level with preconcentration times of less than five min. The reproducibility and recovery were found to be satisfactory as can be seen in Tables III and IV. In addition, the FIA sub-system could be used as screening method to determine the total concentration of heavy metal present in a large number of samples thus avoiding the continuous use of the chromatograph for routine analysis. At present the method is been applied to the determination of these analytes in real water samples. The strong interference from iron calls for a complex systematic study in order to overcome it.

Acknowledgement

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