ELSEVIER

Journal of Chromatography A, 883 (2000) 127–136

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Improved scheme of chelation ion chromatography with a mixed eluent for the simultaneous analysis of transition metals at μg l^{-1} levels

Xiaojing Ding, Shi-fen Mou*, Kena Liu, Yan Yan

Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China

Received 2 February 2000; accepted 7 March 2000

Abstract

An improved scheme of chelation ion chromatography (CIC) system and a mixed eluent for the simultaneous determination of transition metals are described. A method based on the improved CIC system and the mixed eluent (PDCA/$Na_2C_2O_4$/LiOH/NaCl) for the analysis of seven metals (Pb^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+} and Mn^{2+}) at μg l^{-1} levels in a single isocratic elution is developed. The optimize conditions which are different from references for analyte concentration and chromatographic separation are studied in detail. D418 chelation resin is used to further reduce values of the reagent blank. The above seven metals are measured at 565 nm using 2-[(5-Bromo-2-Pyridyl)-Azo]-5-Diethyl-AminoPhenol(5-Br-PADAP) as the post-column derivatizing reagent. Detection limits range from 0.3 to 12 μg l^{-1} when 4 ml of sample is pre-concentrated. The results of real sample analysis are satisfactory.

Keywords: Seawater; Transition metals

1. Introduction

The determination of trace metals at μg l^{-1} levels in complex matrices remains one of the most challenging areas of analytical chemistry. For it is often the case that most analytical techniques are readily applicable to simple matrices, many may fail when applied to samples in complex matrices. The conventional ion chromatography (IC) of metal cations as a strong competition from atomic spectrometric technique usually makes separations vulnerable to big changes in ionic strength. Injection of a sample with a high concentration of alkali and alkaline earth metals, for example can overload the column and seriously degrade or destroy the separation of the metal cations, and thus, seriously restrict the use of IC for trace metal determinations in a number of areas. However, there is a solution to this problem, that is, to use Chelation Ion Chromatography (CIC). To avoid confusion, it must be pointed out that the CIC method discussed in this article does not refer to the High-performance Chelating Ion-exchange Column technique (HPCIC) defined by Jones and Nesterenko [1] which involves chelating exchangers impregnated into high-performance substrate, but rather to a method involving two or three columns with valve switching arrangements. Although HPCIC

*Corresponding author. Fax: +86-10-6292-3552.
E-mail address: shifenm@mail.rcess.ac.cn (S.-f. Mou)

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00313-7

is also an efficient method to determine trace elements in complex matrices and has been reviewed [1], it is beyond the scope of our discussion.

CIC as a new method was first proposed by Siriraks et al. [2] for the simultaneous determination of trace metals at μg l^{-1} levels in complex matrices. He claimed that a higher cross-linked resin containing iminodiacetic acid functional groups was suitable for use at elevated pressure, providing reliable and reproducible analysis of complex matrices, and then successfully applied it to the simultaneous determination of Fe^{3+}, Cu^{2+}, Ni^{2+}, Zn^{2+} and Mn^{2+} in seawater; Fe^{3+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Mn^{2+} and Fe^{2+} in bovine liver. It is considered a very powerful technique that makes possible the determination of trace and ultra trace metals in complex matrices that have proven to be difficult or impossible to analyze by conventional ion chromatography or atomic spectrometry. It receives increasing attention due to its major advantage of ease of automation and analyte concentration and matrices elimination.

The reported procedure of this method requires sample loading on a chelating column (Dionex MetPac CC-1), followed by a rinse step with 2 mol l^{-1} ammonium acetate buffer (pH 5.5) to selectively elute alkali and alkaline earth metals, subsequent transfer of the concentrated transition metals to a second concentrator column–a high-capacity sulfonated cation-exchange cartridge (Dionex TMC-1) with 0.5 mol l^{-1} nitric acid, and then the TMC-1 column is converted to the ammonium form by using 0.1 mol l^{-1} ammonium nitrate (pH 3.5) while retaining the metals. Finally, the TMC-1 column is placed in the chromatographic stream and the metals are separated.

The configuration for automation of the CIC method first proposed by Siriraks et al. could simultaneously perform the CIC process on two sets of chelator and concentrator columns and permitted a new sample to be determined every 25 mm. It involved one gradient pump (GPM) for delivering separation eluate to the analytical column, three single reciprocating piston pumps (DQP) for delivering several reagents during the sample pre-concentration and matrix elimination steps, ten air-activated metal free valves and eight solenoid valve controls for switching reagents, samples and columns. Some efforts to simplify the configuration and at the same time to determine transition metals as more as possible in one injection have been made [3–9]. The main improvements were listed in Table 1. References [3–6] used a flow diagram which involved two advanced gradient pumps (AGP), two DQP and five valves to analyze transition metals. $Fe^{3}+$, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+} (or Cd^{2+}) in 0.086 mol l^{-1} $MgCl_2$, urine and tissue etc. were analyzed by using an oxalic acid-based (Ox) or an pyridine-2,6-dicarboxylic acid-based (PDCA) eluent [3]. Caprioli and Torcini [4] determined Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+} in seawater. However, Pb^{2+} and Cd^{2+} could not be determined. Because the Pb^{2+}-PDCA complex was more stable than the corresponding Pb^{2+}-PAR (4-(2-pyridylazo) resorcinol) complex and Cd^{2+} was difficult to separate from Co when 6 mmol l^{-1} PDCA was used as the eluent. Mou et al. [5] analyzed lanthanide metals in geological samples. Shotyk and Potthast [6] ever used two separator

Table 1
The main modifications to the configuration for CIC system

Pumps (number)	Valves (number)	Columns (number)	Metals determined	Sample	Refs.
4	10	5	Fe^{3+}, Cu^{2+}, Ni^{2+} Zn^{2+}, Co^{2+}, Mn^{2+}	seawater	[2]
3	5	3	Fe^{3+}, Cu^{2+}, Ni^{2+} Zn^{2+}, Co^{2+}, Mn^{2+}	0.086 mol l^{-1} $MgCl_2$	[3]
3	4	3	Pb^{2+}, Cu^{2+},Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}	drinking water	[9]
3	3	3	Pb^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+} Mn^{2+}	seawater	our present work

columns (CS5 analytical column) in series to improve peak separation. When 6 mmol l^{-1} PDCA/0.4 mol l^{-1} NaOH was used as the eluent, Fe^{3+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+} and Mn^{2+} in coral skeletons could be simultaneously determined. When 6 mmol l^{-1} PDCA/0.4 mol l^{-1}NaOH/2 mmol l^{-1} Na_2SO_4/15 mmol l^{-1}NaCl (pH4.4) was used as the eluent, Pb^{2+} could be analyzed along with the above seven transition metals.

However, The principle disadvantage of using two separator columns is the increased time required per analysis (10 min for concentrating the sample and almost 40 min for analytical chromatography)., Lu et al. further improved the configuration which involved two AGP, one DQP and four valves, and then determined lanthanide metals in agricultural [7] and geological [8] samples; Pb^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+} and Cd^{2+} in drinking water by a concentration gradient of Ox eluent while eliminating high concentrations of alkali, alkaline earth metals, iron (III) and aluminum [9]. However, Mn^{2+} co-eluted with Cd^{2+} and Mn^{2+} had to be oxidized to MnO_4^- prior to ion chromatographic separation, Moreover, the reductive component would reduce permanganate to Mn^{2+}, and thus inevitably influenced the quantitative analysis of Cd^{2+}. Cardellicchio et al. [10] ever used one GPM, two DQP and two valves to determine Pb^{2+} and Cd^{2+} in seawater while eliminating high concentrations of alkali and alkaline earth metals. But the other transition metals could not be determined due to the fact that 0.075 mol l^{-1} H_2SO_4/0.1 mol l^{-1} HCl/0.1 mol l^{-1}KCl was used as the eluent on an IonPac CS10A column. Motellier and Pitsch [11] used an inert 625 pump, one DQP and two columns (Dionex MetPac CC-1 chelating column and CS5 analytical column) to transfer the six concentrated transition metals (Fe^{3+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+}) directly onto the analytical column to accomplish the quantitative analysis. However, the high calcium concentration did not allow the quantitative analysis of Fe^{3+}. In a word, using the above CIC methods, Pb^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+} and Mn^{2+} in complex matrices could not be simultaneously determined with a relative simple flow diagram of the CIC system.

The purpose of our present work is to accomplish the simultaneous determination of Pb^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+} and Mn^{2+} in complex matrices by utilizing the CIC method, and at the same time, to further simplify the flow diagram of the CIC system. The simplified diagram involves one advanced gradient pump (AGP), one isocratic pump, one DQP and three air-activated valves. A method based on the mixed eluent first proposed in our previous work [12] combined with the improved CIC system is established. To validate the simplicity and applicability of the improved scheme of the CIC system, a standard mussel sample was analyzed. As a further application, seawater collected from Lianyun Harbour in Southeast of China was analyzed without any additional sample pre-treatment and the results were satisfactory.

2. Experiment

2.1. Instrumentation

Chromatographic analysis were performed on a metal-free Dionex-4000i ion chromatography (Dionex, Sunnyvale, CA, USA), which included one advanced gradient pump (AGP), one isocratic pump, one pneumatic controller for post-column reagent addition (RDM), one eluent degas module (EDM) and a VDM-2 variable wavelength absorbance detector at 565 nm; a MetPac CC-1 chelation column (50×4 mm I.D., packed with styrene-based macroporous 12% cross-linked iminodiacetate-functionalized chelating resin, the particle size is 20 μm and the capacity of the resin is about 0.9 mequiv.); a TMC-1 concentrator column (25×3 mm I.D. containing fully sulfonated PS-DVB cation-exchange resin with a high capacity of 2.2 mequiv.); a Dionex IonPac CG5A guard column (50×4 mm LD.) and an JonPac CS5A analytical column (250×4 mm I.D., 9 μm bead diameter, ethylvinyl benzene-functionalized with both quaternary ammonium and sulfonate functional groups) were used to separate the heavy and transition metals. A 4-ml injection loop was used. All measurements were made at room temperature (30°C±2) and all samples were filtered through a 0.45-μm filter prior to injection. In all cases, injection of the sample was done at least in triplicate.

Data collection and the operation of all components in the system were controlled by Dionex AI-450 chromatographic software interfaced via an

ACI-2 advanced computer interface to a 80486 based computer.

2.2. Reagents and standards

All reagents were analytical grade unless specified otherwise. All solutions were prepared with de-ionized water throughout. Ammonium acetate, lithium hydroxide monohydrate, sodium oxalate, sodium hydroxide, amino acetic acid, 2-[(5-Bromo-2-Pyridyl)-azo] -5-diethylaminophenol (5-Br-PADAP), ethyl alcohol (Peking Chemical Works, Peking, China), sodium chloride (Peking Shunghuan Reagent Factory, Peking, China), sodium nitrate (Peking Hongxing Chemical works, Peking, China), acetic acid (Peking Yili Fine Chemical Institute, Peking, China), sodium pyrophosphate (Peking Xingjin Chemical works, Peking, China) TritonX-100 (Carl Roth KG Chemische Fabrik Karlsruhe). Nitric acid (Peking Chemical Reagent Institute, Peking, China), ammonium hydroxide (Peking Yili Fine Chemical Institute, Peking, China), perchloric acid (Peking Nan Shangou Chemical works, Peking, China) and pyridine-2,6-dicarboxylic acid (PDCA, Aldrich, USA) were of chromatographic grade.

Iminodiphosphate-based chelation resin (D418, 50–100 mesh) is from the Chemical Plant of Nankai University, Tianj in, China).

Working standard solutions were prepared daily by serial dilution of stock standard solutions of each metal containing 1000 mg l^{-1} (National Research Center for Certified Reference Materials, Peking, China).

All standards and samples were stored in acid washed (10% nitric acid) polyethylene bottles.

2.3. Eluents and post-column reagent solution purification of D418 resin

Approximately 150-g D418 resin was placed in a 1-l polyethylene bottle and then cleaned with a 4%NaOH solution→de-ionized water→2.0 mol l^{-1} HNO_3→de-ionized water in turn. Finally, the resin was stored in 500 ml of 2.0 mol l^{-1} ammonium acetate and 20 mmol l^{-1} sodium pyrophosphate buffer solution (pH 5.5) until required.

Eluents: E_1 0.18 mol l^{-1} $NaNO_3$, pH=3.34; E_2, 2.0 mol l^{-1} NH_4AC and 20 mmol l^{-1} $Na_4P_2O_7$.10 H_2O, pH5.5; E_3, 0.5 mol l^{-1} HNO_3 E_4, 3 mmol l^{-1} PDCA/3 mmol l^{-1} $Na_2C_2O_4$/4.2 mmol l^{-1} LiOH/10 mmol l^{-1}NaCl. Flow-rate: 1.0 ml min^{-1}. E_5, 'carrier' acid, 0.1 mol l^{-1} HNO_3.

The post-column reagent comprised 0.3 mmol l^{-1} 5-Br-PADAP/0.95% Triton X-100 (w/v)/0.066 mol l^{-1} amino acetic acid/0.067 mol l^{-1}NaOH/0.12 mol l^{-1}NaCl (pH=12). Flow-rate: 0.45 ml mm^{-1}.

2.4. Sample preparation

A 2.0-g standard mussel sample (The Research Center for Eco-Environmental Sciences of Chinese Academy of Sciences, Peking, China) was weighed and placed into a 100-ml ground-glass flask followed by a few drops of de-ionized water to moist the sample. 10 ml concentrated nitric acid was added. The flask was capped and placed in the darkness overnight. A funnel was put above the flask, then carefully heated on an electrothermic plate to almost dryness. Two ml perchloric acid was added until a white fume emerged. A 2 ml volume of concentrated nitric acid was again added until the brown fume completely disappeared which indicated that the organic compound had been digested completely. Then the solution was evaporated to almost dryness and diluted to 25 ml. Lianyun Harbour seawater was adjusted to pH 2 with HNO_3. All sample solutions were directly injected after filtered through a 0.45-μm filter.

2.5. Experimental procedure

A detailed flow diagram of the chelation ion chromatographic system is shown in Fig. 1. The programming of time and events for column, valve, and reagent switching is outlined in Table 2. The eluent containers are pressurized with nitrogen to 5 p.s.i.. (34 kPa). When the operation starts, the sample is flushed by the acid carrier (0.1 mol l^{-1} HNO_3) to a mixing tee, where it is buffered on-line with a sodium pyrophosphate–ammonium acetate buffer before entering the MetPac CC-1 column. None of the anions and monovalent cations is retained. Alkaline earth metals, iron(III) and aluminum are selectively removed by a sodium pyrophosphate–ammonium acetate buffer. Then, the retained metals

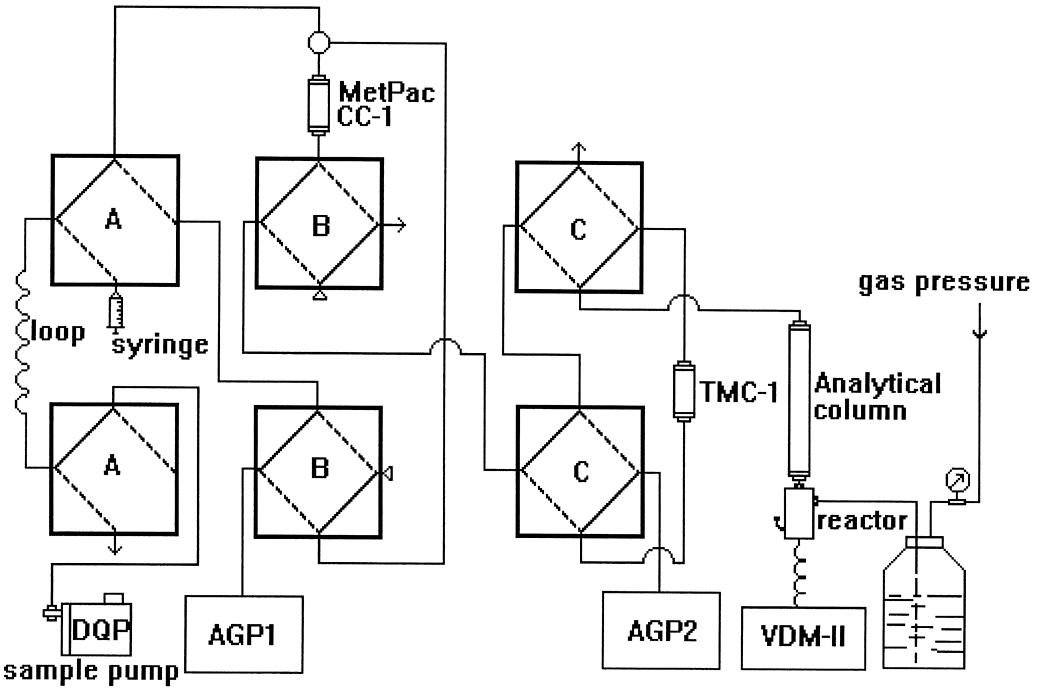

Fig. 1. Scheme of the chelation ion chromatographic system.

are transferred to a TMC-1 column by 0.5 mol^{-1} HNO_3 and rinsed with a 0.18 mol^{-1} $NaNO_3$ solution to convert the TMC-1 column from the hydrogen form to the sodium form. Finally, the TMC-1 column is linked to the CS5A analytical column. AGP2 delivers PDCA eluent to separate metals which are

Table 2
Chelation concentration and matrix elimination operating conditions for the analysis of heavy and transition metals (AGP1 program)

Time (min)	E_1 (%)	E_2 (%)	E_3 (%)	V_A[a]	V_B	V_C	Flow rate (ml min^{-1})	Events
0.0	0	100	0	on	off	on	1.0	sample buffers online with
4.0	0	100	0	on	off	on	1.0	$NH_4AC+Na_4P_2O_7$
4.1	0	100	0	off	off	on	2.0	$NH_4AC+Na_4P_2O_7$ removes
6.0	0	100	0	off	off	on	2.0	$Ca+^{2+}Mg$, Fe^{3+}, Al^{3+}
6.1	0	0	100	off	on	off	2.0	acid removes metals from
9.0	0	0	100	off	on	off	2.0	MetPac CC-1 to TMC-1
9.1	100	0	0	off	on	off	2.5	TMC-1 is changed from
17.0	100	0	0	off	on	off	2.5	acid form to sodium form
17.1[b]	0	100	0	off	off	on	2.0	metals are eluted by PDCA
22.0	0	100	0	off	off	on	2.0	from TMC-1 to CS5A
22.1	0	100	0	off	off	on	0.0	column. At the same time, $NH_4AC+Na_4P_2O_7$ wash the MetPac CC-1 column
34.0	0	100	0	off	off	on	1.0	metal analysis finished and is ready for the next run.

[a] V: valve.
[b] Begin sample analysis. On: real line connected; off: dotted line connected. Sample pump flow-rate: 1.7 ml min^{-1}.

detected after post-column reaction with 5-Br-PADAP at 565 nm.

3. Results and discussion

3.1. Chromatographic separation, matrix elimination and analyte concentration

Since the optimized conditions of chromatographic separation, matrix elimination and analyte concentration have been discussed in detail [2–11], in this study, only the conditions which were different from the references [2–11] are described.

Our previous work [12] first tests that Pb^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+} and Mn^{2+} are well separated on a Dionex CS5A column with a mixed eluent (3 mmol^{-1} PDCA/3 mmol^{-1} $Na_2C_2O_4$/4.2 mmol l^{-1} LiOH/2 mmol l^{-1} Na_2SO_4) by conventional IC method. So, the present work is to employ the CIC method for analyzing the above seven metals in complex samples, and what's more, to simplify the configuration of the CIC system as far as possible by fully utilizing the mixed eluent. For the mixed eluent plays a key role in the separation of the above seven metals especially the separation of Co^{2+} and Cd^{2+} in a single isocratic elution, and thus the reduction of a air-activated valve on the basis of reference [9] is possible 0.03 mmol l^{-1} PDCA was ever added to 10 mmol l^{-1} citric acid eluent [13]. It was found that the retention times of transition metals were halved and under these conditions elution was governed mainly by ion-exchange mechanisms. If high levels of PDCA were incorporated in the eluent, the complexation processes were favored. So, in our previous work, 3 mmol l^{-1} PDCA together with 3 mmol l^{-1} $Na_2C_2O_4$ was first used as the eluent. It was found that the above seven metals in the mixed eluent had a better separation and shorter elution time. $Na_2C_2O_4$ not only helped to elute the metals, but it was also used as an inorganic modifier because it could improve the separation of Co^{2+} and Cd^{2+}. Besides $Na_2C_2O_4$, Na_2SO_4 was also used as an inorganic modifier at the same time. It was also found that Co^{2+} and Cd^{2+} could not be well separated when $Na_2C_2O_4$ and Na_2SO_4 were used separately as the modifier.

Under the above mixed eluent, Fe^{3+} and Al^{3+} did not interfere with the determination of metals due to the presence of $Na_2C_2O_4$ in the eluent. However, Fe^{2+}co-elute with Mn^{2+} and it could be eliminated by adding 30% H_2O_2 to the analyzed samples. Using the present CIC method, Fe^{3+} in the reagent might interfere with the determination of Mn^{2+}, NaCl as an inorganic modifier was thus used instead of 2 mmol l^{-1} Na_2SO_4 to try to overcome the Mn^{2+}/Fe^{2+} co-elution problem. It was found that Mn^{2+} and Fe^{2+} were baseline-separated when the concentration of NaCl≥10 mmol^{-1}; the eluents for matrix elimination and concentration were 2.0 mol l^{-1} NH_4AC+20 mmol^{-1} $Na_4P_2O_7$.10 H_2O and 0.5 mol l^{-1} HNO_3 respectively; the 'carrier' acid was 0.1 mol^{-1} HNO_3 and the concentration of $NaNO_3$ ranged from 0.05 mol l^{-1} to 0.2 mol^{-1} to convert the TMC-1 column from the hydrogen form to the salt form.

The conditions (0.5–1.0 mol l^{-1} H^+) that are effective for eluting the concentrated metal ions from the MetPac CC-1 column are not compatible with the eluent system of the analytical column (IonPac CS5A) [2,3]. A high capacity cation-exchange concentrator must be used to retain the metal ions as they are eluted from the MetPac CC-1 column. The TMC-1 contains a fully sulfonated cation-exchange resin with sufficient capacity (0.3 meq) to retain the metal ions under elution conditions from the MetPac CC-1. The TMC-1 interfaces the high capacity chelating column with the low capacity analytical column. Before the TMC-1 can be switched in line with the analytical stream, it must be converted from the acid (H^+) form to the ammonium (NH_4^+) form by using ammonium nitrate, pH3.5. The ammonium nitrate eluent selectively and effectively elutes the hydrogen ion, but due to its dilute concentration (0.1 mol l^{-1}) and optimal pH (3.5), the transition metals are not eluted. Lu [9] first utilized 0.18% $NaNO_3$ to convert the TMC-1 column from the acid form to the sodium form, otherwise, the peaks of Pb^{2+}, $Cu^{2+}$and Cd^{2+} would be within a big negative peak and could not be determined accurately. Our present work find that under the improved scheme and the mixed eluent conditions, 0.18 mol l^{-1} $NaNO_3$ was appropriate to convert the TMC-1 column to salt form. It was also found that the retention time of metals decreased with the increasing concentration of $NaNO_3$. The experiment showed that the optimum

concentration for NaCl and $NaNO_3$ was 10 mmol l^{-1} and 0.18 mol l^{-1}, respectively. If the concentration of $NaNO_3$ was lower than 0.1 mol l^{-1}, the Pb^{2+} peak would fall into the large negative peak of water. If the concentration of $NaNO_3$ was higher than 0.2 mol^{-1}, detection sensitivity of the metals would decrease. The possible reason was that high concentration of $NaNO_3$ would elute the metals from the TMC-1 concentrating column. The optimized conditions for chromatographic separation, matrix elimination and concentration are listed in Experimental Section 2.3. A typical chromatogram of a synthetic standard solution is shown in Fig. 2. All individual metal peaks are well separated. Even though there are 5 mg ml^{-1} calcium, 5 mg ml^{-1} magnesium, 2 mg ml^{-1} aluminum and 2 mg ml^{-1} iron in the solution, they do not interfere with the separation and the determination of transition metals.

3.2. Post-column reagents and spectrophotometric detection

The choice of post-column reagent is crucial for the improvement of detection limits. 5-Br-PADAP as a post-column reagent was ever compared with PAR [14]. It was found that the former increased the sensitivity for Cu^{2+}, Cd^{2+}, Zn^{2+} and Mn^{2+} and 0.3 mmol l^{-1} 5-Br-PADAP/0.8% Triton X-100/0.5 mol l^{-1} NH_4OH/0.3 mol l^{-1} $NaHCO_3$ (pH 10.5) was optimum condition for determination [9,14]. Our previous study also showed that 5-Br-PADAP formed stable metal complexes with metal ions. Its concentration had a great influence on the color development. When its concentration was increased, the background absorbance also increased thus resulted in signal-to-noise ratios. The presence and the concentration of Triton X-100 were also important in order to increase the solubility and stability of the complex. pH also influenced color development; high pH increased the ionization of 5-Br-PADAP, thus increased its complexing capabilities. However, the background absorbance would be high also and provoked hydrolysis of metals. Some ligands such as F^-, Cl^-, Br^-, I^-, tartaric acid and sulfosalicylic acid etc. that are smaller than the color-producing reagent can be used as a second ligand (L_2) to form metal-5-Br-PADAP-L_2 complex [15,16]. They can make the metals more easily to chelate with 5-Br-PADAP than that with PDCA and thus enhance the detection sensitivity of metals. So, from the above mentioned conditions, 0.3 mmol l^{-1} 5-Br-PADAP/0.95% Triton X-100 (w/v)/0.066 mol l^{-1} amino acetic acid/0.067 mol l^{-1}NaOH/0.12 mol l^{-1}NaCl (pH 12) was used as the post−colunm reagent.

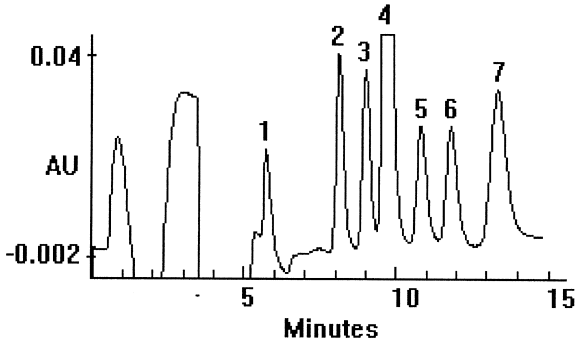

Fig. 2. Chromatogram of metals in a synthetic standard solution. Chromatographic conditions are listed in Table 1 and Section 2.3. Detection is at 565-nm. Peaks: 1, Pb (3 mg l^{-1}); 2, Cu (0.1 mg l^{-1}); 3, Ni (0.3 mg l^{-1}); 4, Zn; 5, Co (0.5 mg l^{-1}); 6, Cd (0.5 mg l^{-1}); 7, Mn (0.4 mg l^{-1}), Fe (2.0 mg ml^{-1}), Al(2.0 mg ml^{-1}), Ca (5.0 mg m^{-1}), Mg (5.0 mg m^{-1}), respectively. Loop volume: 41.4 μl.

3.3. System blank

In order to reduce the Zn^{2+} levels in the blank, be sure that the eluent bottle caps have a white TFE (tetrafluoroethylene) seal and not a black rubber seal [3]. But we only got a black rubber seal available. So, after the columns were washed with 0.2 mmol l^{-1} oxalic acid for 3 h (flow-rate 1.0 ml min^{-1}). The system was rinsed with 200 ml of de-ionized water. The columns were removed and the flow path was washed again with 6.0 mol l^{-1} HNO_3 for 3 h. Finally, the system was again rinsed with de-ionized water. Care was taken to minimize reagent and sample contamination during preparation and handling, trace metal impurities still existed on the system blank especially Zn^{2+} exhibited the greatest contamination level. These impurities could be further reduced by placing the cleaned D418 chelation resin into the eluent containers which contained 2.0 mol l^{-1} NH_4AC+20 mmol l^{-1} $Na_4P_2O_7$.10 H_2O and

0.18 mol l^{-1} $NaNO_3$, respectively. However, copper, zinc and manganese are the most common metal contaminants in the available reagents and cannot be entirely eliminated, while a small amount of lead and nickel may also be observed. Fig. 3 shows a typical level of background contamination in a blank run.

To assure the validity of the analytical data, the evaluation of the analytical blanks using chelation concentration was performed and several blanks (n= 7) were analyzed. The average values of the blank, when 4 ml of de-ionized water was pre-concentrated, were 12, 0.43, 1.2 and 1.0 μg l^{-1} for Pb^{2+}, Cu^{2+}, Ni^{2+} and Mn^{2+}, respectively. The observed RSDs were 5.8, 0.7, 4.4 and 5.2% for lead, copper, nickel and manganese, respectively. There was no memory effect when a blank was run immediately after a sample with a high concentration of metals. The zinc was not determined because of its high level found in the blank during the analytical procedure. Cobalt and cadmium peaks did not occur on the blank chromatogram.

3.4. Linearity and detection limits

In this work, an optimized method was developed. It gave rise to sharp peaks with good peak separation and excellent calibration curves. Table 3 illustrated that all metals had good linearities with correlation coefficients greater than 0.9989. The detection limits, defined as the signals corresponding to three times the noise levels were also calculated. The RSD based on >10× the detection limits, was found to be in the range of 3.3–5.6%. The data confirmed that the precision of this method was good. A large loop lowered the detection limits and did not have any detrimental effects on peak symmetry. However, the system blanks would also increase accordingly. So, 4 ml loop was used in this study.

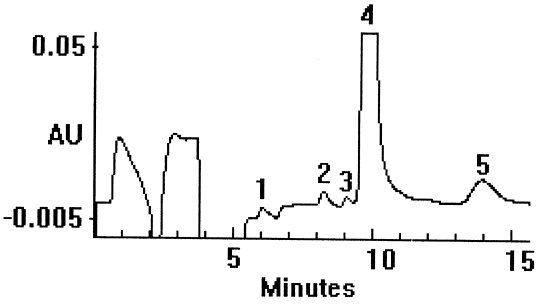

Fig. 3. Typical level of background using chelation concentration. Peaks: 1, Pb^{2+}(12 μg l^{-1}), 2, Cu^{2+}(0.43 μg l^{-1}), 3, Ni^{2+} (12 μg l^{-1}), 4, Zn^{2+}, 5, Mn^{2+} (1.0 μg l^{-1}). Loop volume: 4 ml.

Table 3
Linear ranges, correlation coefficients, RSD values and detection limits for heavy and transition metals

Metal	Concentration range (ng ml^{-1})	Correlation coefficient	RSD (%)	Detection limit (ng ml^{-1})
Pb^{2+}	12–200	0.9989	5.4	12
Cu^{2+}	0.4–20	0.9999	5.5	0.4
Ni^{2+}	1.2–30	0.9994	3.3	1.2
Co^{2+}	1.0–50	0.9999	3.3	0.3
Cd^{2+}	1.0–50	0.9999	5.6	0.3
Mn^{2+}	0.8–40	0.9997	3.9	0.8

3.5. Samples

Samples of standard mussel and seawater collected from Lianyun Harbour were analyzed. All the samples were digested and acidified with 0.5 mol l^{-1} HNO_3 to pH 2, then diluted with de-ionized water and directly injected to the IC system through a 0.45-μm filter. The results were summarized in Table 4. The concentration of Pb^{2+} and Ni^{2+} in mussel sample was below the detection limits and, therefore, could not be determined. The concentration of Co^{2+} was close to its detection limit and it was masked by the high level of Zn^{2+}, thus it could not be determined either (Fig. 4.). Spike studies were carried out in the mussel sample with an ion standard solution prior to the acid digestion step for the determination of recovery rates. They were in the range from 82.2% to 98.8% with R.S.D% ranging from 2.7% to 6.2%. In Lianyun Harbour seawater, only Cu^{2+} and Mn^{2+} were determined (Fig. 5). Pb^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+} and Cd^{2+} could not be determined due to the high system blank, which prevented the use of a large loop volume. Good

Table 4
Analysis of real samples

Sample		Content					
		Pb	Cu	Ni	Co	Cd	Mn
Mussel	Certified ($\mu g\ g^{-1}$)	1.96±0.1	7.7±1.0	1.03±0.14	0.94±0.06	4.5±0.6	10.2±1.8
	determined ($\mu g\ g^{-1}$)	ND[a]	6.9	ND	ND	4.4	8.9
	Spiked ($\mu g\ g^{-1}$)	25	5	7.5	6.2	6.2	10
	Recovery (%)	85.2	91.9	83.6	87.0	98.8	93.8
	R.S.D (%)	6.2	2.7	4.1	5.3	3.3	6.0
Lianyun Harbour seawater	IC ($14\ \mu g\ l^{-1}$)	ND	0.68	ND	ND	ND	73
	Spiked ($14\ \mu g\ l^{-1}$)	40	8	12	10	10	16
	Recovery (%)	90.0	94.0	89.4	92.8	99.0	93.0
	R.S.D (%)	5.5	2.6	2.7	4.4	2.3	4.6

[a] Not detected.

recovery results for all of the metals were obtained and listed in Table 4.

4. Conclusions

The present improved scheme of chelation ion chromatography system combined with the mixed eluent was applicable and easy to be operated. It could be used to analyze Pb^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+} and Mn^{2+} at $\mu g\ l^{-1}$ levels in complex matrices in a single isocratic elution. Large quantities of salty matrix interference were completely eliminated while the analytes were concentrated. A standard mussel sample was analyzed and the IC values obtained were in good agreement with the certified values.

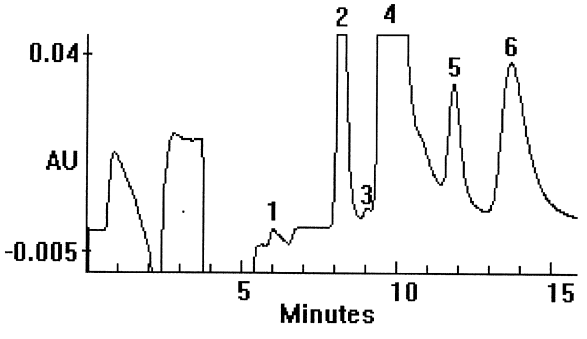

Fig. 4. Chromatogram of the standard mussel sample. Sample solution was HNO_3 (pH 2) then diluted 1:50 (v/v) prior to injection. Chromatographic conditions are the same as Fig. 4. Loop volume: 4 ml. Peaks: 1=Pb, 2=Cu ($6.9\ \mu g\ g^{-1}$), 3=Ni, 4=Zn, 5=Cd ($4.4\ \mu g\ g^{-1}$), 6=Mn ($8.9\ \mu g\ g^{-1}$).

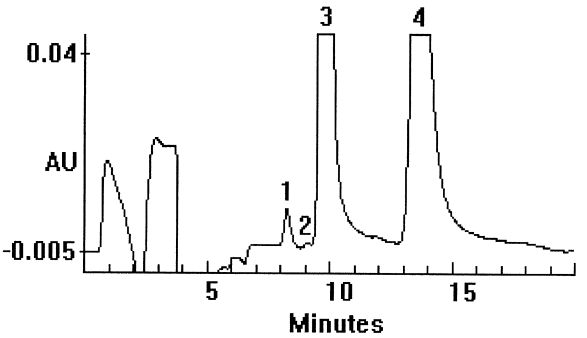

Fig. 5. Chromatogram of the Lianyun Harbour seawater. Chromatographic conditions are the same as Fig. 4. Loop volume: 4 ml. Peaks: 1=Cu ($0.68\ \mu g\ l^{-1}$), 2=Ni, 3=Zn, 4=Mn ($73\ \mu g\ l^{-1}$).

References

[1] P. Jones, P.N. Nesterenko, J. Chromatogr. A 789 (1997) 413.
[2] A. Siriraks, H.M. Kingston, Anal. Chem. 62 (1990) 1185.
[3] Dionex, No. 25, Technical Note (1992).
[4] S. Caprioli, S. Torcini, J. Chromatogr. 640 (1993) 365.
[5] S.F. Mou, A. Siriraks, J.M. Riviello, sepu (Chinese Journal) 12 (3) (1994) 166.
[6] W. Shotyk, I.I. Potthast, J. Chromatogr. A 706 (1995) 167.
[7] H.T. Lu, S.F. Mou, Y. Yan, F. Liu, K.A. Liu, S.Y. Tong, J.M. Riviello, Talanta 45 (1997) 119.
[8] H.T. Lu, S.F. Mou, Y.W. Hou, F. Liu, K.A. Li, S.Y. Tong, Z.L. Li, J. Liq. Related Technol. 20 (19) (1997) 3173.
[9] H.T. Lu, S.F. Mou, Y. Yan, S.Y. Tong, J.M. Riviello, J. Chromatogr. A 800 (1998) 247.
[10] N. Cardellicchio, S. Cavalli, J.M. Riviello, J. Chromatogr. 640 (1993) 207.
[11] S. Motellier, H. Pitsch, J. Chromatogr. A. 739 (1996) 119.
[12] X.J. Ding, S.F. Mou, K.N. Liu, A. Siriraks, J. Riviello, Anal. Chim. Acta. 407 (2000) 319.
[13] G. Bonn, S. Reiffenstuhl, J. Chromatogr. 499 (1990) 669.
[14] N. Cadellicchio, S. Cavalli, P. Ragone, J.M. Riviello, J. Chromatogr. A 847 (1999) 251.
[15] F.S. Wei, Y.R. Zhu, F. Yin, Anal. Lett. 14 (A4) (1981) 241.
[16] D.A. Johnson, T.M. Florence, Talanta 22 (1975) 253.