Sequential Injection Chromatography with post-column reaction/derivatization for the determination of transition metal cations in natural water samples

Burkhard Horstkotte *, Patrícia Jarošová, Petr Chocholouš, Hana Sklenářová, Petr Solich

Department of Analytical Chemistry, Faculty of Pharmacy, Charles University in Prague, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic

A B S T R A C T

In this work, the applicability of Sequential Injection Chromatography for the determination of transition metals in water is evaluated for the separation of copper(II), zinc(II), and iron(II) cations.

Separations were performed using a Dionex IonPAC^{TM} guard column (50 mm $\times$ 2 mm i.d., 9 μm). Mobile phase composition and post-column reaction were optimized by modified SIMPLEX method with subsequent study of the concentration of each component. The mobile phase consisted of 2,6-pyridinedicarboxylic acid as analyte-selective compound, sodium sulfate, and formic acid/sodium formate buffer. Post-column addition of 4-(2-pyridylazo)resorcinol was carried out for spectrophotometric detection of the analytes' complexes at 530 nm.

Approaches to achieve higher robustness, baseline stability, and detection sensitivity by on-column stacking of the analytes and initial gradient implementation as well as air-cushion pressure damping for post-column reagent addition were studied.

The method allowed the rapid separation of copper(II), zinc(II), and iron(II) within 6.5 min including pump refilling and aspiration of sample and 1 mmol HNO_3 for analyte stacking on the separation column. High sensitivity was achieved applying an injection volume of up to 90 μL. A signal repeatability of $< 2\%$ RSD of peak height was found. Analyte recovery evaluated by spiking of different natural water samples was well suited for routine analysis with sub-micromolar limits of detection.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Non-separating flow techniques (FT) are powerful tools for the automation of analytical procedures, especially where high sample throughput, high repeatability, and the avoidance of sample contamination during processing are very important such as in ambient monitoring of trace compounds.

This is due to the (computer-)controlled handling of all solutions in a closed tubing network or "manifold", continuous/discontinuous system cleaning by a carrier flow, and precise timing between all procedure steps such as mixing and detection.

With respect to solution handling, mixing conditions, flow pattern, and system configuration, different non-separating FT can be distinguished, with Flow Injection Analysis (FIA) [1], Sequential Injection Analysis (SIA) [2], and Multi-Syringe Flow Injection Analysis (MSFIA) [3] being the ones with the highest impact on this work.

The combination of FT for sample handling and preparation with liquid chromatography (LC) is advantageous because despite all its

potentials, the most definite disadvantage of all FT is the difficulty of differentiation or gradual separation of analytes.

Generally, selective reagents and detection techniques, interference masking, or observance of the reaction kinetic allow discrimination of the analyte from sample matrix components or enable multicomponent analysis by changing the reagent or the reaction conditions appropriately. Further separations, such as gas diffusion or extraction, allow enhancing the method's selectivity. However, without coupling FT with chromatographic or electrophoretic separations, mono- or oligo-analyte methods with potential cross-sensitivity to other compounds are typical [4,5].

In this context, the development of monolithic columns [6] and their integration into FT analyzer systems allowing low-pressure chromatographic separations [7] was a milestone for the development of more versatile analytical procedures based on FT. Consequently, the combination of the different FT led to the proposal of Sequential Injection Chromatography (SIC) [8] and later Multi-Syringe Chromatography (MSC) [9] and Flow Injection Chromatography (FIC) [10].

The separation performance even using gradient approaches [11] is similar or inferior to HPLC, mainly because the applicable pressure and column length in SIC are limited. The main

advantages of SIC and the related technique are the possibility of automated in-system sample treatment such as sample clean-up [12], the flexibility of operation such as the use of reagents for analyte derivatization or on-column stacking, the ease of method set-up, and the lower cost and transportability of the analyzer system enabling its use for monitoring and field operation.

Applications of SIC and related techniques used mostly commercial silica-based reverse-phase monolithic columns and were focused mainly on the separation of organic analytes. Lately, monolithic columns available with new functionalities and core-shell columns [13] are opening the field of application and operation of SIC [14]. Two comprehensive reviews can be found elsewhere [15,16].

While separation of metal cations with post-column addition of either chromogenic or chemiluminescence-generating reagents are state-of-the-art in HPLC [17,18], in FT, multi-parameter methods are mainly based on the use of ion-selective reagents.

Due to the lack of commercial monolithic columns with cation-exchange functionality, either dynamic coating of reverse phases [19] or permanent modification [20] have been used to enable cation separation.

In the present work, the potential and application of a cross-linked divinylbenzene stationary phase modified by a bilayer of anion- and cation-exchange functionalized latex for low-pressure separation of transition cations in SIC are studied. As an alternative to monolithic columns, only the guard column of an HPLC column assembly [21] was used and rapid and low-pressure separation of copper(II), zinc(II), and iron(II) was achieved.

Firstly in SIC, post-column addition of a chromogenic reagent was applied to enable selective analyte detection [22]. For this reason, an additional syringe pump was used to provide a confluent flow of an alkaline solution of 4-(2-pyridylazo)resorcinol (PAR) that is typically used as a chromogenic reagent for sensitive detection of transition metal cations by the formation of red-colored complexes [17,23–25].

2. Material and methods

2.1. Reagents

All solutions were prepared with demineralized quality water (resistivity > 18 MΩ cm) and all reagents were of quality "for analysis" and purchased from Sigma Aldrich (Prague, Czech Republic).

For the preparation of the chromogenic reagent, the following aqueous stock solutions were prepared: 50 mmol L^{-1} of 4-(2-pyridylazo)resorcinol (PAR) in 100 mmol L^{-1} NaOH, 4.0 mol L^{-1} ammonium hydroxide, and 1.0 mol L^{-1} NaHCO$_3$. For the preparation of the mobile phase, the following stock solutions were prepared: 250 mmol L^{-1} 2,6-pyridinedicarboxylic acid (PDCA) in 750 mmol L^{-1} NaOH, 2 mol L^{-1} formic acid, and 500 mmol L^{-1} Na$_2$SO$_4$. In addition, 2.0 mol L^{-1} NaOH was used for pH adjustment of mobile phase and post-column reagent. Aliquots of the stock solutions of PAR and PDCA were stored in the dark at $-18\,^{\circ}$C until use. All working solutions were prepared daily.

Further stock solutions of the transition metal cations of interest, namely Co(II), Cu(II), Fe(II), and Zn(II) of 1 mmol L^{-1}, were prepared in 10 mmol L^{-1} nitric acid. Nitrate salts were used for Co(II) and Zn(II) and sulfate salts for Cu(II), and Fe(II). Standard solutions were prepared daily with 4 mmol L^{-1} of nitric acid.

Freshwater samples were used to evaluate the applicability of the method. They were acidified with a surplus of nitric acid (12 mmol L^{-1}) to overcome the buffer capacity of dissolved bicarbonates and carbonates, achieving a final pH of about 2 being similar to the standard solutions. The samples were used unfiltered but after settling of sediments overnight.

2.2. Instrumentation

The analyzer configuration is schematically shown in Fig. 1. All experiments were carried out on a Sequential Injection Chromatography system (SIChrom™) from FIAlab company (Bellevue, WA, USA). It integrated a medium pressure, biocompatible piston pump (PP) from Sapphire Engineering (IDEX Corporation, Oak Harbor, WA, USA) of 4 mL total dispense volume (3 inlets) and an eight-port selection valve (SV1, type Cheminert, 12U-0484H, 5000 psi) from VICI (Valco Instruments Co. Inc., Houston, TX, USA).

A second selection valve of the same type (SV2) and an additional syringe pump (SP, type Cavro XL) from Tecan (San Jose, CA, USA) equipped with a 2.5 mL glass syringe were used to enable piston pump refilling and addition of the post-column reagent, respectively. All instrumentation was controlled via RS232 interface. The second outlet of the PP was connected to a pressure safety valve (PSV) of 500 psi release threshold.
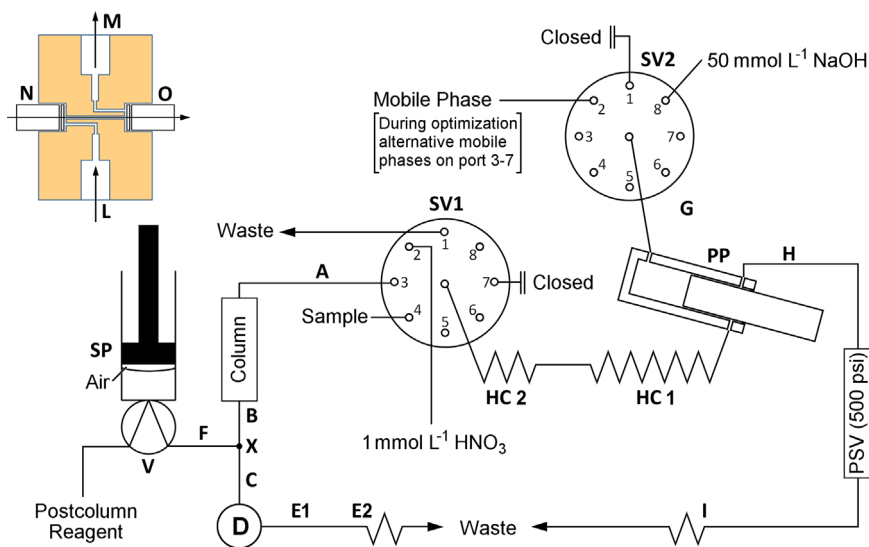

Fig. 1. Scheme of the SIC analyzer system. D: detection flow cell, HC1 and HC2: holding coils (150 cm, 0.8 mm i.d. and 30 cm 0.5 mm i.d., PEEK), PP: piston pump, PSV: pressure safety valve, SP: syringe pump with air bubble, SV1 and SV2: selection valves, V: syringe head valve, X: confluence (T-connector). Dimensions of PEEK tubing with 0.25 mm i.d. A: 25 cm, B: 5 cm, C: 15 cm, E1: 5 cm. Dimensions of FEP tubing (0.8 mm i.d.), E2 & I: 100 cm, G: 30 cm, H: 30 cm. Detail drawing: detection flow cell with flow inlet (L) and outlet (M) and optical fiber connections (N & O).