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Synthesis and application of a functionalized resin for flow injection/F AAS copper determination in waters

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Abstract

This paper reports the development of a new strategy for low-level determination of copper in water samples by using a flow-injection system coupled to solid-phase extraction (SPE) using flame atomic absorption spectrometry (F AAS) as detector. In order to preconcentrate copper from samples, a minicolumn packed with a styrene-divinylbenzene resin functionalized with (*S*)-2-[hydroxy-bis-(4-vinyl-phenyl)-methyl]-pyrrolidine-1-carboxylic acid ethyl ester was used and the synthesis procedure is described. System operation is based on the on-line retention of Cu(II) ions at pH 9.0 ± 0.2 in a such minicolumn with posterior analyte elution with $2\,\mathrm{mol\,l^{-1}}$ HCl directly to the F AAS nebulizer. The influence of several chemical (sample pH, buffer concentration, HCl eluent concentration and effect of the ionic strength) and flow (sample and eluent flow rates and preconcentration time) variables that could affect the performance of this system were investigated as well as the possible interferents. At optimized conditions, for 2 min of preconcentration time (13.2 ml of sample volume), the system achieved a detection limit of $1.1\,\mu\mathrm{g\,l^{-1}}$, a R.S.D. 1% at $20\,\mu\mathrm{g\,g\,l^{-1}}$ and an analytical throughput of $25\,\mathrm{h^{-1}}$, whereas for 4 min of preconcentration time (26.4 ml of sample volume), a detection limit of $0.93\,\mu\mathrm{g\,l^{-1}}$, a R.S.D. 5.3% at $5\,\mu\mathrm{g\,l^{-1}}$ and a sampling frequency of $13\,\mathrm{h^{-1}}$ were reported.

Keywords: Copper; Functionalized resin; F AAS; Solid-phase extraction; Flow-injection analysis

1. Introduction

In the last few years, many efforts have been spent in the analytical chemistry area for developing methodologies with suitable productivity and sensitivity, especially in the water analysis field. In this context, FIA systems have been successfully coupled to solid-phase extraction (SPE) for the preconcentration/separation of metallic cations with several types of no modified solid sorbents, such as C_{18} [1] polyurethane foam [2–5], activated carbon [6], activated alumina [7,8] or fullerene [9]. Several advantages over common batch procedures, such as lowest reagent consumption, easy operation and highest analytical throughput explain the growing interest in this coupling. Besides, errors due to sample losses or contamination are strongly reduced, since sample handling is minimized during all analytical procedures.

Additionally to the use of no modified sorbents, several works can be found in the literature regarding to the use of solid-phases loaded with specific reagents to improve on-line SPE procedures [10–19]. It is important to remark that, in all these cases, the reagent was only adsorbed onto solid-phase surface.

Another approach that has been employed in on-line SPE systems is the application of functionalized resins synthesized in the own laboratory. In this case, as the same way that for loaded solid-phases, specific ligands are chosen to

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enhance the performance of the system. However, resins in which reagents are incorporated to the polymeric structure of the material present, in general, highest lifetime, because the ligand molecule is not easily leached from the column during preconcentration and elution steps. On the other hand, these resins seems to have only moderate capacity value, which according to Kumar et al. [20] can be overcome by an extensive functionalization of an appropriately cross-linked polymer.

In this scenario, Lemos et al. developed two systems for flow-injection determination of copper [21] and cobalt [22] by F AAS using Amberlite XAD-2 resin functionalized with 3,4-dihydroxybenzoic acid and *R*-nitroso salt, respectively. The system developed for copper determination achieved an enrichment factor of 33 (120 s preconcentration time) while that developed for cobalt presented enrichment factors of 79 and 223 depending on the preconcentration time employed (60 or 180 s, respectively). Both methodologies were validated by the analysis of certified materials of organic samples and by the analysis of spiked water samples.

Naghmush et al. [23] functionalized cellulose with phosphonic acid and quaternary amines aiming the speciation of chromium by an on-line procedure employing F AAS as detection technique. According to the authors, cellulose functionalized with phosphonic acid was found to be superior to preconcentrate Cr(III), while cellulose with quaternary amines group showed better performance in the sorption of Cr(VI). The same research group compared the performance of cellulose functionalized with phosphonic acid with several other sorbents for the on-line determination of lead by F AAS [24].

Alonso et al. [25] developed an automated system for the separation–preconcentration of Cd from saline waters by ET AAS. In order to achieve this goal, the authors placed a minicolumn packed with silica gel functionalized with 1,5-bis(di-2-pyridyl)methylene thiocarbohydrazine in the autosampler arm, dispensing the eluted sample directly to the autosampler cup. More recently, Zougagh et al. [26] employed the same solid-phase in a flow system for on-line solid-phase manganese preconcentration and determination by ICP OES. The influence of several variables that could affect the performance of the system was evaluated by using a multivariate approach based on a Doehlert design.

An Amberlite XAD-4 resin modified with *N*-hydroxyethylethylenediamine was used by Hirata et al. [27] for on-line determination of total iron and Fe(II) by chemiluminescence with brilliant sulfoflavine and hydrogen peroxide. The system was able to preconcentrate iron from sea water allowing the determination of the analyte at nmol l^{-1} level.

In this work, an on-line preconcentration system was developed for the determination of Cu(II) in waters using a minicolumn packed with a polystyrene-divynilbenzene resin functionalized with (*S*)-2-[hydroxy-bis-(4-vinylphenyl)-methyl]-pyrrolidine-1-carboxylic acid ethyl ester. The synthesis and characterization of the chelating resin was followed by the study of the chemical and hydrodynamic parameters of the analytical system. The developed method has been applied to the trace determination of copper in water samples.

2. Experimental

2.1. Apparatus

All measurements were performed in a Perkin-Elmer Analyst 100 flame atomic absorption spectrometer (Norwalk, CT, USA) equipped with a copper hollow cathode lamp furnished by own Perkin-Elmer. The equipment was operated at standard conditions recommended by the manufacturer. The wavelength was set at 324.8 nm, the lamp current was adjusted to 10 mA and a lean-blue air–acetylene flame was employed (air, 13 l min^{-1}; acetylene, 2.5 l min^{-1}). Transient peaks obtained were registered with a x–y graphic recorder supplied by Equipamentos Cientificos do Brasil (Curitiba, Brazil). The pH measurements and adjustments were carried out in an Analyser 300 (São Paulo, Brazil) apparatus equipped with a combined glass electrode. A Gilson Minipuls 3 (Villiers le Bel, France) peristaltic pump provided with flexible PVC tubes was used to propel the solutions. A Rheodyne 5041 (Cotati, CA, USA), six-port rotary valve was used to select preconcentration or elution steps. The system manifold was built of PTFE tubes with 0.5 mm i.d. and connections made of PEEK.

ET AAS measurements were carried out with a Hitachi Z-8200 (Tokyo, Japan) polarized Zeeman-effect spectrometer equipped with a copper hollow cathode lamp coupled to a Hitachi SSC-300 autosampler. The instrumental conditions were those suggested by the manufacturer of the equipment (lamp current = 7.5 mA; wavelength = 324.8 nm; spectral bandwidth = 1.3 nm). Argon was used as purge gas together with pyrolytical-coated tube-type graphite furnace. Background signals-correction was made by employing a polarized Zeeman effect-based corrector and the temperature program was that previously employed by our group for the determination of copper in aqueous solutions (Table 1) [28], which allowed the determination of copper in the range 5–20 μg l^{-1}. Palladium nitrate chemical modifier (10 μl of a 1000 mg l^{-1} solution) was employed in the analysis of all samples.

Table 1
Temperature program employed for the determination of copper by ET AAS (adapted from Ref. [28])

Temperature stage	Temperature (°C)	Time (s)	Argon flow rate (ml min^{-1})
Drying	80–90	20	200
Drying	90–100	20	200
Drying	100–120	20	200
Drying	120–140	10	200
Pyrolysis	1000	30	200
Atomization	2500	10	0
Cleaning	2550	4	200

2.2. Reagents and solutions

The solutions used throughout the experimental work were prepared by using water purified in a Milli-Q Water System from Millipore (Milford, MA, USA). Reagents with analytical grade or higher were used as received. Standard solutions of Cu(II) were daily prepared by suitable dilution of 1000 mg ml^{-1} stock solution, which was prepared by dissolving 2.5434 g of $CuSO_4 \cdot 8H_2O$ in 1000 ml of 0.2 mol l^{-1} HNO_3 solution. This solution was titrimetrically standardized.

The eluent solution, 2 mol l^{-1} HCl, was prepared by diluting 167 ml of concentrated acid supplied by Merck (Darmstadt, Germany), in a volume of water enough to made up 1000 ml.

Borate buffer solution with 0.5 mol l^{-1} concentration and pH 9.0 was prepared by dissolving 15.45 g of boric acid in around 400 ml of water. Afterwards, KOH was added until solution achieved desired pH. Then, the volume was filled up to 500 ml, and the pH was measured again.

2.3. Resin synthesis and minicolumn preparation

2.3.1. Resin synthesis

The preparation of the resin utilized in this work can be divided in three steps following reactions previously cited in the literature [29,30]: (i) preparation of the intermediate (pyrrolidine-1,(*S*)-2-dicarboxylic acid-1-ethylesther-2-methylester); (ii) synthesis of the monomer (*S*)-2-[hydroxy-bis-(4-vinyl-phenyl)-methyl]-pyrrolidine-1-carboxylic acid ethyl ester; (iii) reaction between polystyrene, divinylbenzene and the monomer to obtain the polymeric functionalized resin. All reaction products were identified and characterized by infrared spectroscopy (IRS), ^{1}H NMR, ^{13}C RMN and/or mass spectrometry (MS).

2.3.1.1. Prepration of the intermediate. In the first step, a reaction between L-proline and ethyl chloroformate was promoted to form the carbamate-esther compound (pyrrolidine-1,(*S*)-2-dicarboxylic acid-1-ethylester-2-methylester), which contains the chelating group to be supported on the resin. The equation representing such reaction is shown in the Fig. 1a.

This reaction was carried out in a dry flask maintained under argon atmosphere by mixing 1.15 g of L-proline, 1.32 g of potassium carbonate and 20 ml of methanol. To this mixture, 2.5 g of ethyl chloroformate was slowly added and, after this, the system was refrigerated until 0 °C. The solution obtained was shaken during 2 h and then deionized water and saturated solution of $NaHCO_3$ were added. At this condition, the intermediate carbamate-ester (substance C) was formed and extracted with three portions of 15 ml of ethyl acetate. These portions were dried with anhydrous Na_2SO_4, and the yellow liquid obtained was distilled at low pressure being obtained 1.90 g of an uncolored liquid of C with a boiling point of 93 °C, that corresponds to a reaction yield of 95%. The identification of the reaction product was performed by infrared spectroscopy, ^{1}H NMR, ^{13}C NMR and MS. Spectral data are: IRS-bands at 2982, 1749, 1703, 1420, 1201 cm^{-1}; ^{1}H RMN ($CDCl_3$, 300 MHz)-δ 4.30 (m, 1H, N–CH–(C)–C), 4.09 (m, 2H, CO_2–CH_2–CH_3), 3.70 (s, 3H, CO–CH_3, rotameters), 3.48 (m, 2H, N–CH_2), 2.18 (m, 1H, um N–CH–(C)–CH_2), 1.93 (m, 3H, um N–CH–(C)–CH_2, N–CH–(C)–CH_2–CH_2), 1.20 (dt, 3H, $J = 7$ Hz, CO_2–CH_2–CH_3, rotameters); ^{13}C RMN ($CDCl_3$, 75.5 MHz)-δ 173.8; 155.3; 61.6; 59.3; 52.5; 46.9; 30.7; 24.3; 15.1; MS-(*m*/*z*) 201 (molecular ion), 142 (100%), 114 (7%), 70 (50%), 128 (13.3%).

2.3.1.2. Synthesis of the monomer. The synthesis of the monomer (substance E, (*S*)-2-[hydroxy-bis-(4-vinyl-phenyl)-methyl]-pyrrolidine-1-carboxylic acid ethyl ester) occurred by reaction between the intermediate prepared in the previous step and the Grignard reagent obtained from reaction between bromine styrene, Mg and I_2 in THF (tetrahydrofuran) medium, as shown in the Fig. 1b.

In order to prepare the Grignard reagent, 2.40 g of powdered Mg together with 1.27 g of I_2 were added to a suitable flask connected to a condenser. During 2 h, this flask was maintained under argon flux and heated in an oil bath. After this, the mixture was left to achieve ambient temperature, 1 ml of THF was added and then refluxed by 1 h. After elapsed this time, 7.9 g of *p*-bromostyrene dissolved in 4.2 ml of THF was slowly added, and the system was maintained in reflux by 1 h more, until the formation of a black precipitate. The suspension formed was agitated until achieve 25 °C and 2.0 g of C, dissolved in 14.3 ml of THF, were added. The mixture obtained was agitated for 1 h, and then 10 ml of purified water were added. The monomer formed was extracted with three portions of 20 ml of ethyl ether, and the organic phase obtained was washed with saturated solution of $NaHSO_3$ and dried with anhydrous $MgSO_4$. A concentration step was carried out by evaporation of the solvent at low pressure, and the oil obtained was purified in a column loaded with silica gel containing 1% (w/v) of triethylamine employing the mixture hexane:ethyl ether as eluent. Working at these conditions, 2.85 g of E were obtained in a form of a pale yellow oil (yield of 76%). The product formed was characterized by ^{1}H RMN ($CDCl_3$, 200 MHz) presenting the following spectral data: δ 7.47 (m, 8H, Ar–H); 6.73 (dd, $J = 10.9$ Hz and $J = 17.6$ Hz, 2H, Ph–CH–CH_2), 5.81 (dd, $J = 17.9$ Hz, 2H, um Ph–CH–CH_2), 5.26 (d, $J = 10.9$ Hz, 2H, um Ph–CH–CH_2), 4.66 (m, 1H, N–CH–(C)–C), 1.84 (m, 2H, NCH_2–CH_2), 1.04 (t, $J = 6.9$ Hz, 3H, rotameters).

2.3.1.3. Synthesis of the functionalized resin. The synthesis of the functionalized resin was performed via free radical initiation with azo-bis-isobutyronitrile (AIBN). The polymerization was carried out in a heterogeneous medium during 30 h at 85 °C and constant agitation. This reaction can be represented by the equation shown in Fig. 1c.

The preparation was done by mixing 0.33 g of polyvinyl alcohol dissolved in 66 ml of water + 3.43 g of divinylbenzene + 4.12 g of styrene + 0.56 g of monomer previously pre-

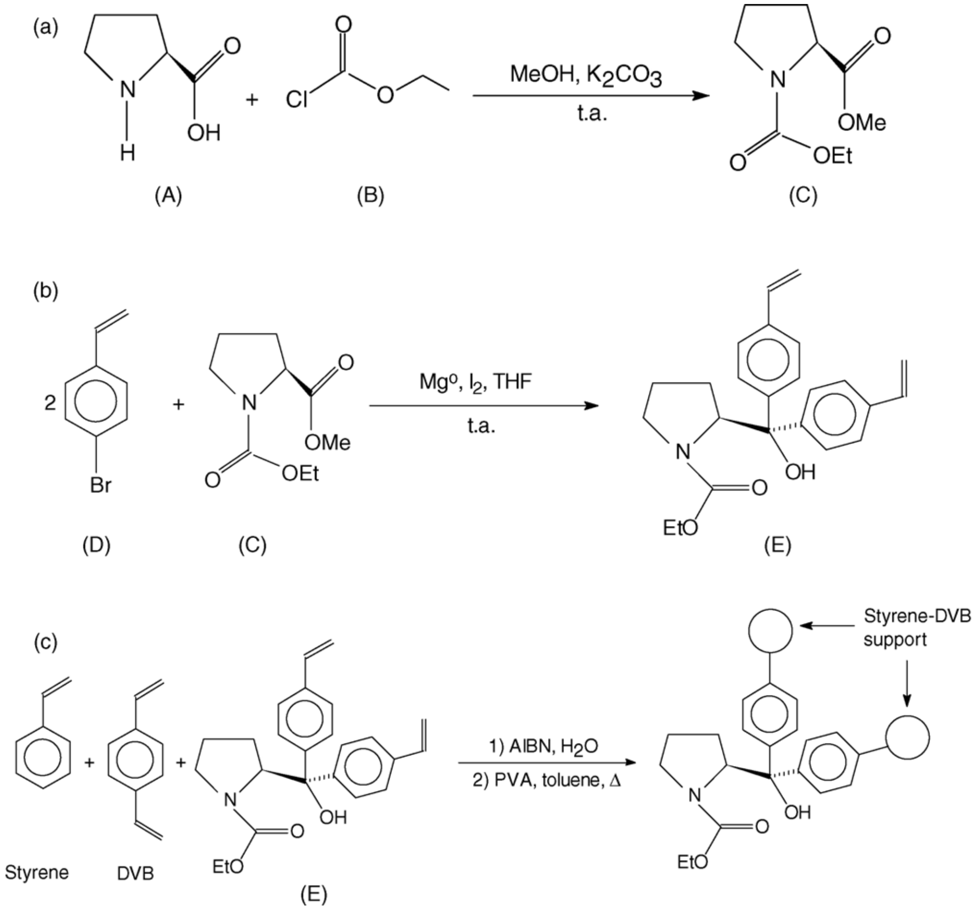

Fig. 1. Scheme of the synthesis of the porous resin. Reactions used in the preparation of the: (a) intermediate; (b) monomer; (c) functionalized porous resin, where (A) L-proline; (B) ethyl chloroformate; (C) intermediate, pyrrolidine-1,(*S*)-2-dicarboxylic acid-1-ethylester-2-methylester; (D) bromostyrene; (E) monomer, (*S*)-2-[hydroxy-bis-(4-vinyl-phenyl)-methyl]-pyrrolidine-1-carboxylic acid ethyl ester; (F) structure proposed for the functionalized resin.

pared + 108 mg of AIBN dissolved in 10 ml of toluene. The mixture obtained was heated to 85 °C in an oil bath under agitation of 400 rpm for 30 h. After elapsed this time, the porous resin (white solid with spherical shape) was separated from the solution by filtration and washed with 100 ml of methanol. The yield obtained with this procedure was 89%, resulting in the formation of 6.71 g of porous resin. The resin was characterized by infrared spectroscopy presenting characteristic bands at 3438, 3024, 1671, 1601 and 1451 cm^{-1}. Special attention was given to the band situated at 1671 cm^{-1} that is a characteristic of the presence of C=O group from carbamates.

2.3.2. Minicolumn preparation

Thirty five milligrams of the resin synthesized as described above was packed in a polyethylene tube with 4.0 cm length × 3 mm i.d. Afterwards, the resin was washed by pumping 10 ml of 2 mol l^{-1} HCl at 2 ml min^{-1}. The excess of acid was removed by passing water through minicolumn until the effluent became nearly neutral.

2.4. Flow-injection system

A schematic diagram of the developed flow system is depicted in Fig. 2. In this system, the sample solution, main-

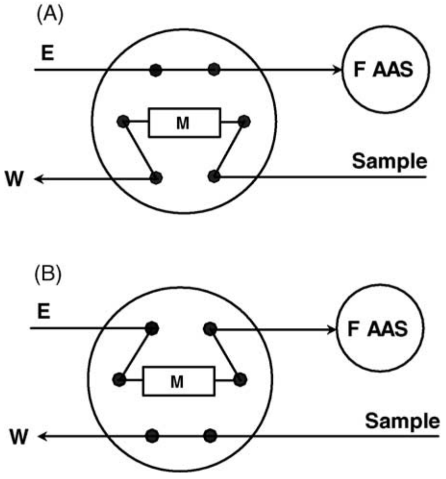

Fig. 2. System manifold for flow-injection preconcentration and determination of copper in water samples using functionalized resin as solid-phase: (A) preconcentration step and (B) elution step; S, sample (pH = 9.0 ± 0.2, 6.60 ml min^{-1}); E, eluent solution, 2 mol l^{-1} HCl (4.9 ml min^{-1}); M, minicolumn (35 mg) containing functionalized resin; F AAS, detector, atomic absorption spectrometer (324.8 nm); W, waste.

tained at pH 9.0, was pumped at 6.6 ml min^{-1} directly to the minicolumn containing 35 mg of functionalized resin. After elapsed a suitable preconcentration time (typically 2 min), the valve was switched, and the eluent solution (2 mol l^{-1} HCl) then percolated through minicolumn at 4.9 ml min^{-1}, desorbing the analyte from the solid-phase owing to a high decrease in pH, what reduced resin complexing capacity. Besides, formation of chloro-complexes of copper helped elution process. Afterwards, the sample plug was driven to the spectrometer, where absorbance measurements were continuously made at 324.8 nm. Thus, transient signals were derived, and peak heights were used as quantitative parameter. After the elution step, which takes about 22 s, the minicolumn is ready for a new preconcentration cycle.

2.5. *Water samples*

Water samples were taken and stored in polyethylene bottles and concentrated nitric acid (ultra pure grade) was added up to a concentration of 2% (v/v). Nitric acid was added to avoid Cu(II) adsorption onto the flask walls. After this, the samples were preserved on ice and taken to the laboratory, where they were filtered, if necessary, with a polycarbonate filtration device. Before analysis, 90 ml of preserved sample were mixed with 10 ml of 0.5 mol l^{-1} borate buffer (pH = 9.0), and the resulting solution was injected into the system.

3. Results and discussion

In order to achieve the best performance for the flow system in terms of analytical throughput and sensitivity the influence of several parameters was investigated. This study was performed taking into account three different groups: (1) study of the influence of chemical variables; (2) the investigation of the effect of flow parameters; (3) evaluation of the possible interferents, including the influence of ionic strength (matrix interference) on system performance.

3.1. *Study of the influence of system variables*

The first parameter investigated was the sample pH. It is well known that complexing capacity of the chelating group supported on the resin is dependent of the pH of the medium due to the presence of proton acceptors, such as nitrogen and oxygen that act as coordination points in the molecule. The influence of the pH sample on the analytical signal was then studied within the range from 6.0 to 10.5. As can be seen in the Fig. 3, the optimum retention was observed within the range between 8.8 and 9.0. When the pH sample increased to values over 10, a progressive decrease in absorbance signals was observed due to possible alkaline hydrolysis of the carbamate-ester group immobilized on the resin, which diminished the retention capacity of the solid-phase, decreasing the analytical signal. On the other hand, at pH under 8.5 and especially at neutral or slightly acidic range, there is a tendency to occur protonation of oxygen and nitrogen atoms present in the resin structure also decreasing the retention capacity of the functionalized solid-phase. Therefore, samples and standards were always prepared with their pH in the range 9.0 ± 0.2. In order to keep sample solution in a suitable pH range, borate buffer solutions with total concentration between 0.010 and 1 mol l^{-1} were tested. Best results were verified for buffer solution with a 0.050 mol l^{-1} total borate concentration and for this reason, this buffer solution was employed in all further experiments.

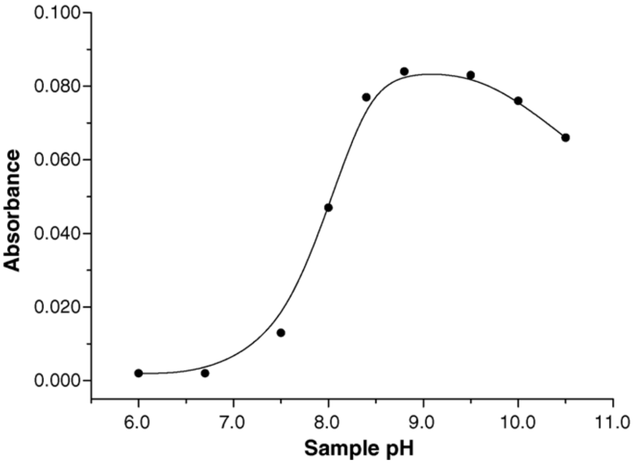

Fig. 3. Influence of pH on absorbance signals derived by the developed methodology; Cu(II), 50 μg l^{-1}; sample flow rate, 5.01 ml min^{-1}; preconcentration time, 2 min; HCl eluent concentration, 4 mol l^{-1} and 4.9 ml min^{-1}.

HCl solution was chosen to desorb copper from the solid-phase, because it provides a strong decrease of pH inside the minicolumn, which reduces the complexing capacity of the chelating group anchored on the resin. Moreover, it can also provide formation of anionic chloro-complexes of Cu(II), favoring the releasing of the analyte from the solid-phase. The study of the best concentration of HCl eluent solution was carried out from 0 (purified water) to 4 mol l^{-1}. Absorbance signals were maximum and constant, when acid concentration was higher than 1 mol l^{-1}, as can be seen in Fig. 4. So, in order to ensure total elution of Cu(II) ions from the minicolumn even for highest concentrations of the analyte, an eluent solution of 2 mol l^{-1} HCl was selected for the method. Also, at this concentration, the time required to the signal returns to the baseline was lower than that observed for other concentrations, which improved the analytical throughput of the system.

In FIA–SPE systems, one of the most important parameters is the sample flow rate, since it regulates the amount of material that pass through the minicolumn. Therefore, the influence of this parameter must be carefully investigated in order to set an optimum sample flow rate that allows a maximum mass transfer from liquid to solid-phase without loss of analytical throughput. So, the effect of the sample flow rate was examined from 3.01 to 8.10 ml min^{-1} and highest ab-

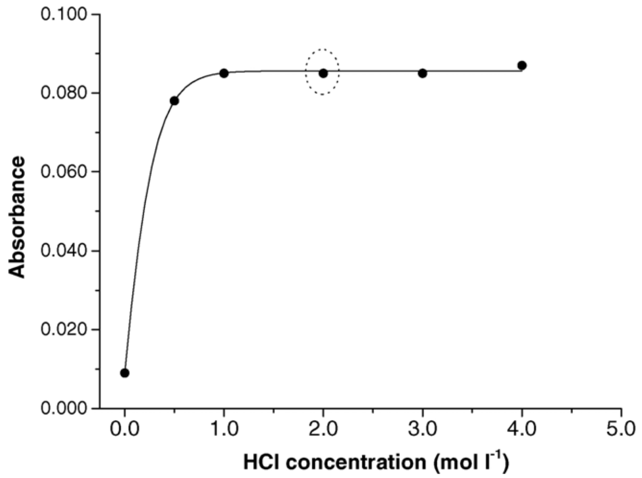

Fig. 4. Influence of concentration of HCl eluent solution on the analytical signal; Cu(II), 50 μg l^{-1}; sample flow rate, 5.01 ml min^{-1}; preconcentration time, 2 min; flow rate, 4.9 ml min^{-1}.

sorbance signals were obtained for 8.10 ml min^{-1}. However, best concentration efficiency, calculated as the ratio between absorbance signal and sample flow rate, was observed at a flow rate 6.60 ml min^{-1}, as can be seen in Fig. 5. This value was then selected for the method, because there was not significant difference (lower than 5%) between sensitivity observed at this condition and those observed at 8.10 ml min^{-1}. The effect of the carrier elution flow rate was also studied in order to achieve an analytical throughput as high as possible. Experiments were carried out by pumping eluent solution at flow rates between 3.01 and 4.90 ml min^{-1}. Best results were verified at 4.90 ml min^{-1}, and this value was selected for the method. Flow rates higher than 4.90 ml min l^{-1} were not tested due to limitations on aspiration rate of spectrometer nebulizer.

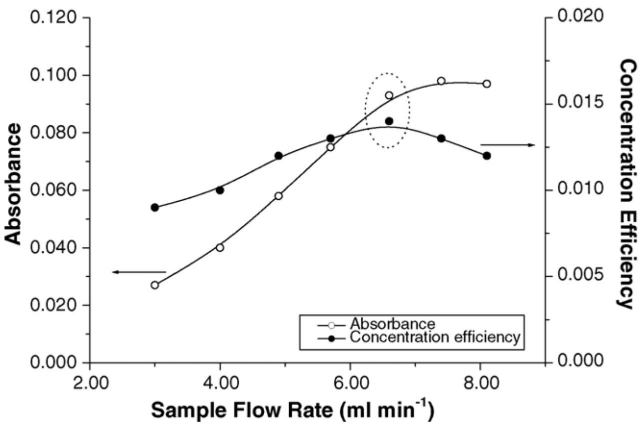

Fig. 5. Influence of sample flow rate on absorbance and concentration efficiency; Cu(II), 50 μg l^{-1}; preconcentration time, 2 min; HCl eluent concentration, 2 mol l^{-1}.

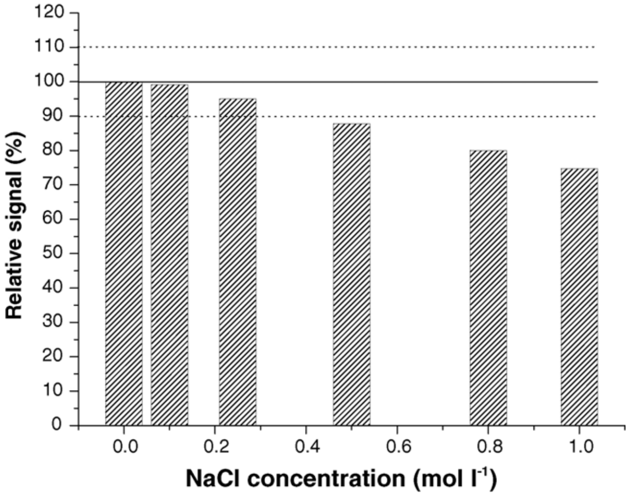

Fig. 6. Effect of ionic strength (as NaCl concentration) on FIA system sensitivity; Cu(II), 50 μg l^{-1}; preconcentration time, 2 min; sample flow rate, 6.60 ml min^{-1}; HCl eluent concentration, 2 mol l^{-1}; HCl eluent flow rate, 4.9 ml min^{-1}.

In time-based FIA–SPE systems, the preconcentration time has noticeable effect on the sensitivity of the procedure, since by increasing this parameter is possible to retain increased amounts of the analyte, allowing its determination in samples with lowest analyte concentrations. However, this process is limited by the effect of minicolumn washing with the own sample or standard. Large sample volumes can result in more severe minicolumn washing, thus diminishing the concentration efficiency. So that, a study about the influence of the preconcentration time on the analytical signal yielded by 50 μg l^{-1} Cu(II) solution was done by varying it from 1 to 5 min. As result, a linear fit was obtained with the following equation: $A = 0.0466\,t$ (min) – 0.0004, $r = 0.997$. This result indicates that in the present case the washing process is negligible up to 5 min preconcentration time, being possible to improve the quantification limits by using higher preconcentration times.

After establishing the best conditions for system operation, the influence of the ionic strength on the retention of Cu(II) by the resin was investigated, since the salinity of water samples can vary in a large degree depending on the origin of the water. For this purpose, the influence of the NaCl concentration on the analytical signal was investigated between 0.1 and 1.0 mol l^{-1}. From the obtained results (Fig. 6), it can be concluded that significant effect was already noted, when NaCl concentration was equal or higher than 0.5 mol l^{-1}, being observed a decreasing in absorbance around 12% at 0.5 mol l^{-1} and 25% at 1 mol l^{-1}.

3.2. Evaluation of the capacity of retention of the minicolumn

In order to evaluate the retention capacity of the functionalized at optimized on-line conditions, a solution containing 1.0 mg l^{-1} Cu(II) was continuously percolated through the minicolumn, and effluent fractions with 5 ml were collected to measure the amount of copper retained by difference. The experiment was stopped, when the amount of Cu(II) ions retained not varied from one fraction to the other in a range of 5%. From the data derived in this experiment, the mini-column containing 35 mg of functionalized resin was able to retain 735 μg of Cu(II), which represents a retention capacity of 21 mg g^{-1}.

3.3. *Interference study*

In spite of inherent selectivity in flame AAS measurements, an interference study was made, once the presence of other cations could decrease copper retention by competition for the active sites of the solid-phase. Then various possible interferent species usually present in waters were investigated in presence of 50 μg l^{-1} Cu(II). Zn(II), Co(II), Ni(II), Mn(II), Pb(II) and Fe(III) were tested in concentrations up to 1000 μg l^{-1}. In these cases, only Fe(III) and Zn(II) caused variation in the Cu(II) signal, when found in concentrations of 1000 μg l^{-1}, being tolerated only up to 500 μg l^{-1}. On the other hand, Ca(II), Mg(II) and Al(III) did not present any effect on Cu(II) signal even when tested at concentration of 50 mg l^{-1}.

4. Features of the proposed system

The proposed methodology was able to produce analytical fits with good linearity in the range 10–200 μg l^{-1}, when a preconcentration time of 2 min was employed. In such condition, a typical equation $A = (0.001 \pm 0.003) + (0.0016 \pm 0.0003)$ [Cu(II) (μg l^{-1})] with correlation coefficient $r = 0.9995$ was derived. In such condition, a sampling frequency of 25 h^{-1} was obtained, and a detection limit of 1.1 μg l^{-1} was achieved by following the procedure recommended by Miller and Miller [31]. At 10 μg l^{-1}, a R.S.D. of 3.9% was observed. Increasing the preconcentration time to 4 min (applied only in the analysis of sea water sample), the analytical fit built up between 5 and 100 μg l^{-1} had the following equation: $A = (0.0029 \pm 0.004) + (0.0033 \pm 0.0006)$ [Cu(II) (μg l^{-1})], with a correlation coefficient $r = 0.9988$. Following same calculations done in the former situation, the detection limit was 0.93 μg l^{-1} and R.S.D. was 5.3% at 5 μg l^{-1}. In this case, a sampling frequency of 13 h^{-1} was observed. Enrichment factors for the system were evaluated as described by Fang [32], e.g., comparing the slopes of analytical fits observed by the proposed methodology with that one derived from direct aspiration of standard solutions of Cu(II) in a concentration range that is possible to achieve reasonable signals. Enrichment factors of 21 and 43 were derived for 2 and 4 min of preconcentration time, respectively.

5. Application of the methodology for the analysis of water samples

In order to check applicability and the accuracy of the proposed methodology for quantitative determinations of copper in waters, some tests were carried out in six different samples that were spiked with 50 μg l^{-1} Cu(II). Table 2 shows achieved results in such analysis, which were always performed in triplicate. As can be seen, good recoveries higher than 90% were reached for the samples with low salinity. On the other hand, in the analysis of a sea water (saline sample) the recovery was 79.5%, evidencing the effect of ionic strength already verified in the study of the influence of this parameter. So on, this sample was analyzed by standard addition method, which provides similar result to that obtained by ETAAS (limit of detection = 0.60 μg l^{-1}), proving that is possible to achieve good accuracy, when standard addition method is employed. Copper was also determined (in triplicate) in all other samples by ETAAS not presenting statistical difference between results obtained, when a paired *t*-test was applied (95% confidence level). All these results are summarized in Table 3.

Table 2
Recovery of copper in the analysis of spiked water samples

Sample	Cu added (μg l^{-1})	Cu found (μg l^{-1})	Recovery (%)
Mineral water I	0	10.2 ± 0.2	–
	50	62.3 ± 1.3	104
Mineral water II	0	17.5 ± 0.8	–
	100	117 ± 3	99.4
Tap water I	0	35.2 ± 0.8	–
	50	88.5 ± 1.9	106
Tap water II	0	16.6 ± 0.3	–
	100	108 ± 5	91.3
Produced water	0	35.2 ± 1.0	–
	50	88.2 ± 1.2	106
Sea water	0	5.07 ± 0.10	–
	50	44.8 ± 1.2	79.5

Determinations were performed in triplicate.

Table 3
Comparison between results (media ± 1 S.D.) obtained by developed FIA system and ET AAS in the analysis of water samples

Sample	FIA (μg l^{-1})	ET AAS (μg l^{-1})[b]
Mineral water I	9.2 ± 0.2	10.0 ± 0.7
Mineral water II	17.5 ± 0.8	20.2 ± 1.7
Tap water I	35.2 ± 0.8	31.6 ± 1.7
Tap water II	16.6 ± 0.3	17.0 ± 1.1
Produced water	35.2 ± 1.0	35.4 ± 2.1
Sea water	5.7 ± 0.10[a]	6.2 ± 0.6

Determinations were performed in triplicate.

[a] In this case, the determination was performed by standard addition method employing preconcentration time of 4 min.

[b] Detection limit for ET AAS = 0.60 μg l^{-1}.

6. Conclusions

The results obtained in this study show that the use of a styrene-divinylbenzene resin functionalized with (*S*)-2-[hydroxy-bis-(4-vinyl-phenyl)-methyl]-pyrrolidine-1-carboxylic acid ethyl ester is suitable for the on-line preconcentration of Cu(II) ions from water samples aiming its determination, at $\mu g\,l^{-1}$ level, by flame atomic absorption spectrometry. The functionalized resin employed in this work presented excellent capacity being able to retain up to 21 mg of Cu/g of resin.

In relation to the flow system, an excellent analytical throughput ($25\,h^{-1}$) was attained, when a 2 min preconcentration time was set. At this condition, a limit of detection of $1.1\,\mu g\,l^{-1}$ was derived, and an enrichment factor of 21 was calculated. Also, such system showed a remarkable advantage: the possibility to employ higher preconcentration times without washing of minicolumn. This characteristic allows the use of high sample volumes, which can increase the potential sensitivity of the methodology.

Finally, preliminary studies indicate that it is possible to expand the applicability of the solid-phase synthesized in this work for the retention of other divalent cations under flow conditions, which can be a promising way to carry out metals on-line determinations with very good analytical features, such as reproducibility, accuracy and sample throughput.

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References

[1] K.A. Tony, S. Kartikeyan, B. Vijayalakshmy, T.P. Rao, C.S.P. Iyer, Analyst 124 (1999) 191.
[2] R.J. Cassella, R.E. Santelli, A.G. Branco, V.A. Lemos, S.L.C. Ferreira, M.S. Carvalho, Analyst 124 (1999) 805–808.
[3] R.J. Cassella, J. Environ. Monit. 4 (2002) 522.
[4] R.J. Cassella, D.T. Bitencourt, A.G. Branco, S.L.C. Ferreira, D.S. Jesus, M.S. Carvalho, R.E. Santelli, J. Anal. At. Spectrom. 14 (1999) 1749.
[5] A.N. Anthemidis, G.A. Zachariadis, J.A. Stratis, Talanta 58 (2002) 831.
[6] R.E. Santelli, M. Gallego, M. Valcárcel, Talanta 41 (1994) 817.
[7] P.P. Coetzee, I. Taljaard, H. Debeer, Fresenius J. Anal. Chem. 336 (1990) 201.
[8] M. Sperling, S. Xu, B. Welz, Anal. Chem. 64 (1992) 3101.
[9] Y.P. Peña, M. Gallego, M. Valcárcel, Anal. Chem. 67 (1995) 2524.
[10] R.J. Cassella, V.A. Salim, L.S. Jesuíno, R.E. Santelli, S.L.C. Ferreira, M.S. Carvalho, Talanta 54 (2001) 61–67.
[11] V.A. Lemos, R.E. Santelli, M.S. Carvalho, S.L.C. Ferreira, Spectrochim. Acta, Part B 55 (1998) 1497–1502.
[12] E. Beinrohr, M. Cakrt, J. Garaj, M. Rapte, Anal. Chim. Acta 230 (1990) 163.
[13] V.A. Lemos, M. de la Guardia, S.L.C. Ferreira, Talanta 58 (2002) 475–480.
[14] S.L.C. Ferreira, J.R. Ferreira, A.F. Dantas, V.A. Lemos, N.M.L. Araújo, A.C.S. Costa, Talanta 50 (2000) 1253–1259.
[15] S.L.C. Ferreira, W.N.L. dos Santos, V.A. Lemos, Anal. Chim. Acta 445 (2001) 145–151.
[16] S.L.C. Ferreira, V.A. Lemos, B.C. Moreira, A.C.S. Costa, R.E. Santelli, Anal. Chim. Acta 403 (2000) 259–264.
[17] S.L.C. Ferreira, V.A. Lemos, R.E. Santelli, E. Ganzarolli, A.J. Curtius, Microchem. J. 68 (2001) 41–46.
[18] V.A. Lemos, S.L.C. Ferreira, Anal. Chim. Acta 441 (2001) 281–289.
[19] V.A. Lemos, W.N.L. Santos, J.S. Santos, M.B. Carvalho, Anal. Chim. Acta 481 (2003) 283–290.
[20] M. Kumar, D.P.S. Rathore, A.K. Singh, Talanta 51 (2000) 1187–1196.
[21] V.A. Lemos, P.X. Baliza, R.T. Yamaki, M.E. Rocha, A.P.O. Alves, Talanta 61 (2003) 675–682.
[22] V.A. Lemos, J.S. Santos, L.S. Nunes, M.B. Carvalho, P.X. Baliza, R.T. Yamaki, Anal. Chim. Acta 494 (2003) 87–95.
[23] A.M. Naghmush, K. Pyrzynska, M. Trojanowicz, Anal. Chim. Acta 288 (1994) 247–257.
[24] A.M. Naghmush, K. Pyrzynska, M. Trojanowicz, Talanta 42 (1995) 851–860.
[25] E.I.V. Alonso, L.P. Gil, M.T.S. Cordero, A.G. Torres, J.M.C. Pavón, J. Anal. At. Spectrom. 16 (2001) 293–295.
[26] M. Zougagh, A.G. Torres, J.M.C. Pavón, Anal. Lett. 36 (2003) 1115–1130.
[27] S. Hirata, H. Yoshihara, M. Aihara, Talanta 49 (1999) 1059–1067.
[28] O.D. Sant'Ana, L.S. Jesuíno, R.J. Cassella, M.S. Carvalho, R.E. Santelli, J. Braz. Chem. Soc. 14 (2003) 728–733.
[29] T.K. Jones, J.J. Mohan, L.C. Xavier, T.J. Blacklock, D.J. Mathre, P. Sohar, E.T.T. Jones, R.A. Reamer, F.E. Roberts, E.J.J. Grabowski, J. Org. Chem. 56 (1991) 763–769.
[30] J.V.B. Kant, M. Perlasamy, Tetrahedron 49 (1992) 5127–5132.
[31] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, third ed., Ellis Horwood PTR Prentice Hall, Chichester, UK, 1993.
[32] Z. Fang, Flow Injection Separation and Preconcentration, John Wiley, Chichester, UK, 1995.