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Application of silica gel organofunctionalized with 3(1-imidazolyl)propyl in an on-line preconcentration system for the determination of copper by FAAS

Edson Luiz da Silva, Amarildo Otavio Martins, Antoninho Valentini, Valfredo Tadeu de Fávere, Eduardo Carasek*

Departamento de Química, Universidade Federal de Santa Catarina, Florianópolis SC 88040-900, Brazil

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Abstract

This study presents a new procedure for the determination of trace levels of copper(II) in an aqueous matrix, through flow injection (FI) on-line preconcentration with a minicolumn packed with silica gel modified with 3(1-imidazolyl)propyl groups. After the preconcentration stage, the analyte was eluted with a HNO_3 solution and determined by flame atomic absorption spectrometry (FAAS). The measurements of the analytical signals were carried out as peak area and peak height with the objective of evaluating the most appropriate absorption measurement for the proposed method. Four procedures to calculate the experimental enrichment factor (EF) were also studied. For a preconcentration time of 90 s the enrichment factors found in this study varied between 19.5–25.8 and 36.2–42.2 for peak area and peak height, respectively. The precision of the proposed method was calculated for a solution containing 20 $\mu g\,l^{-1}$ of Cu(II), when 11.2 ml of solution was preconcentrated ($n = 7$), and their respective relative standard deviation (R.S.D.) values were 1.2 and 1.4% for peak area and peak height, respectively. The detection limits obtained were 0.4 and 0.2 $\mu g\,l^{-1}$ of Cu(II) for peak area and peak height, respectively, with a preconcentration time of 90 s. The on-line preconcentration system accuracy was evaluated through a recovery test on the aqueous samples and analysis of a certified material.

Keywords: FAAS; On-line preconcentration; Organofunctionalized silica gel

1. Introduction

The quantification of metals in low concentrations levels ($\leq \mu g\,l^{-1}$) comprises one of the most important targets in analytical chemistry. This interest is also demonstrated in different areas such as biology and medicine. The atomic spectrometry techniques are extensively employed for the quantification of metal species [1]. In particular, FAAS has been one of the most applied techniques, for the determination of inorganic elements in a variety of samples [2]. However, a preconcentration stage is frequently required, when this technique is employed for the determination of metal ions in low concentrations [3]. In this regard, many preconcentration procedures have been developed for the enrichment of trace metals in a diversity of matrices.

The solid phase extraction (SPE) and liquid–liquid extraction are widely applied for the separation and preconcentration of metals among a variety of methods. The first presents some advantages over the other, such as, lesser waste generation, lesser matrix effect, availability and easy recovery of the solid phase, achievement of higher preconcentration factors, easy adaptation of solid phase in a minicolumn coupled to a continuous flow preconcentration system and it does not generally require the use of toxic solvents [3,4]. Another important advantage of this technique is the possibility of using a relatively simple detection system, such as FAAS rather than techniques without flame, which require more sophisticated equipment [5].

FI on-line separation and preconcentration, with minicolumns containing an appropriate adsorbent, has been a much used method to increase sensitivity and selectivity in the analytical determination of trace metals. This method offers some very favorable features in relation to batch systems, such as: higher sample throughput, better efficiency and enrichment reproducibility, low reagent and samples consumptions, lesser risk of contamination and simple automated operation. Currently, many FI on-line preconcentra-

* Corresponding author. Tel.: +55-48-3319219; fax: +55-48-3319711.
E-mail address: carasek@qmc.ufsc.br (E. Carasek).

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tion systems have been coupled with the FAAS technique, due to the low cost of this equipment and its high analytical velocity [6,7].

In recent years, a variety of organofunctional molecules have been chemically bound to or immobilized on various solid supports. This new chelating solid phase is frequently applied in trace metal separation and preconcentration systems. Among the most used supports is silica gel, since the immobilization of analytical reagents on its surface is relatively simple, especially when compared with the immobilization of reagents on organic polymer supports [8]. Moreover, it shows faster metal ion-exchange kinetics, excellent swelling resistance in different solvents, and a good mechanical, thermal and chemical stability [9]. There are several recent reports on using functionalized silica gel for metal enrichment. The silica gel surface has been modified with various chelating ligands such as herbicide 4-amino-3,5,6-trichloropiconilic acid [10] and 2,4-dichlorophenoxyacetic acid [11], resacetophenone [3], 1,8-dihydroxyanthraquinone [12], 8-hydroxyquinoline [13], 1,10-phenanthroline [14], benzimidazole [15], and other chelating agents.

The silica gel functionalized with 3(1-imidazolyl)propyl groups was synthesized and used as adsorbent material in a glass column, in order to preconcentrate metal ions from ethanol solution [16]. In another study, cobalt(II) phthalocyanine was immobilized on modified silica gel with 3-*n*-propylimidazole. The resulting silica was incorporated into a carbon paste electrode by Fujiwara and Gushikem [17]. This material was shown to be efficient in the electrocatalysis of oxalic acid oxidation. In our laboratory, the silica gel organofunctionalized with 3(1-imidazolyl)propyl, packed into a minicolumn, was applied in a FI on-line preconcentration system. The copper(II) ions in an aqueous matrix were retained by the active sites of the adsorbent material. After the preconcentration stage, the analyte was quantified by FAAS. In order to investigate the most adequate absorption measurement for the proposed method, optimum chemical and flow variables, analytical properties of merit and copper determination of the aqueous samples were obtained as peak area and peak height, since some studies mentioned in the literature, on FI on-line preconcentration systems coupled with FAAS, have carried out the measurements of the analytical signal as peak area [2,4,6,18],whereas the majority, as peak height [5,7,19–28]. Four procedures to calculate the experimental enrichment factor (EF) with the two absorption measurements were also studied.

2. Experimental

2.1. Instrumentation

A Varian Model SpectrAA 50 flame atomic absoption spectrometer, with an air–acetylene flame, was used for copper(II) determination. A copper hollow cathode lamp was run under the conditions recommended by the manufacture. Also the wavelength, slit width and burner height had conventional values. The aspiration rate of the spectrometer was 6 ml min^{-1}. A 320 Mettler Toledo pH meter was used to adjust the pH of solutions. The main flow system components were an Ismatec-IPC peristaltic pump with eight channels and provided with Tygon® tubes, a flow manifold with four three-way solenoid valves, polyethylene tubing (0.8 mm i.d.), sorbent minicolumn and a Y-shaped connector. Solenoid valves were controlled by a microcomputer running software written in Quick Basic 4.5.

2.2. Reagents and solutions

Ultrapure water from a Milli-Q water purification system (Millipore) was used to prepare all solutions. All chemicals were of analytical grade and were used without previous purification. The laboratory glassware was kept overnight in a 10% (v/v) nitric acid solution. Before the use, the glassware was washed with deionized water and dried in a dust free environment.

Working standard solutions of copper(II) were all freshly prepared by appropriate dilution from stock solution (standard for atomic absorption from Carlo Erba). Acetate buffer solution was prepared by mixing 2 mol l^{-1} acetic acid and 2 mol l^{-1} acetate sodium solution in an adequate ratio. Sörensen buffer solution was prepared by mixing 0.084 mol l^{-1} Na_2HPO_4 and 0.067 mol l^{-1} KH_2PO_4 in an adequate ratio. Tris buffer solution was prepared by dissolving 6 g of tris(hidroxymethyl) aminometane in 1 l of water. Nitric acid solutions (Merck) used as eluent in flow system was prepared by appropriate dilution with water from the concentrated acid.

2.3. Preparation of chemically modified silica

The procedure for the organofunctionalization of the silica gel with 3-(imidazolyl)propyltrimethoxysilane groups was carried out as described in the literature [17]. Initially, the silica gel was activated by reflux in 6 mol l^{-1} HCl for 24 h. The silica was then washed with deionized water until it tested negative for chloride using the silver nitrate method. The silica was dried in an oven at 150 °C before the reaction. The immobilization of the organic molecule on the silica surface occurred in two stages. In the first stage, 2 g of imidazol and 1 ml of 3-chloropropyltrimethoxysilane were added to a three necked round bottom flask, containing dry toluene. This solution was refluxed for 24 h, producing 3-(imidazolyl)propyltrimethoxysilane. In the next stage, the 3-(imidazolyl) propyltrimetoxysilane was reacted with 13 g of activated and dried silica gel. The mixture was refluxed in toluene anhydride for 24 h. The whole experiment was carried out under a nitrogen atmosphere. The resulting silica was washed with toluene anhydride, acetone, ethanol and ethanol in water

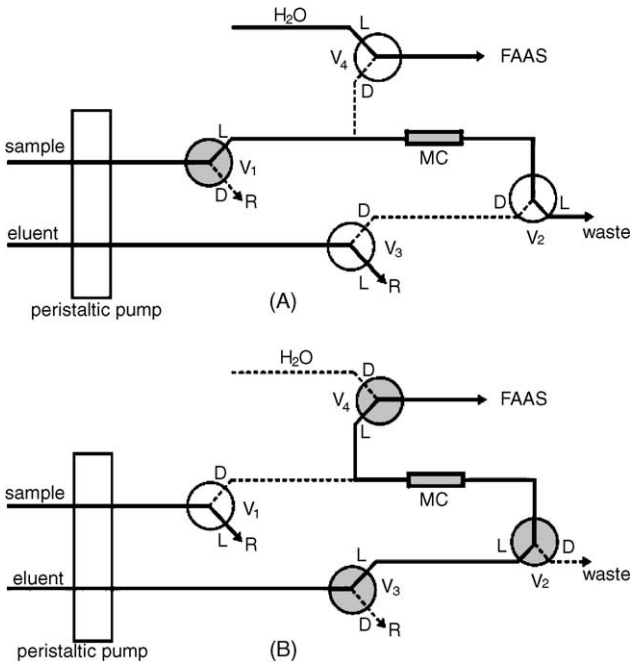

Fig. 1. On-line preconcentration system. (A) preconcentration stage and (B) elution stage. V: valve, L: open way, D: closed way, MC: minicolumn containing adsorbent, R: sample or eluent back stream, hatched circle: valve on and white circle: valve off.

(50% v/v). The solid material was dried at 80 °C under vacuum.

2.4. On-line preconcentration system

The flow manifold is shown schematically in Fig. 1 and it was made up of a peristaltic pump fitted with Tygon® tubes, four three-way solenoid valves and a minicolumn packed with 0.13 g of sorbent material, and it was coupled to a flame atomic absorption spectrometer. The minicolumn with 58 mm of length and i.d. = 3 mm was sealed on both ends with small glass wool beds to prevent material losses. The performance of the minicolumn was stable during all experiments. A qualitative analysis was carried out in order to observe whether the silica organofunctionalized is soluble at pH 10. The assay was done by the colorimetric standard method [29] using ammonium molybdate, and the characteristics for yellow complex was not observed. This indicates that the material is very stable even under rough changes of pH conditions.

The flow system was operated in a time-based mode. In the sample loading step (Fig. 1A), the valve V_1 is initially activated and the others are turned off, so that the sample or standard solution was continuously pumped through the minicolumn where ion exchange takes place and the efluent flows towards waste. In the elution step (Fig. 1B), after sample loading the valve V_1 is turned off and the V_2, V_3 and V_4 are activated. A nitric acid solution was percolated through the minicolumn in reverse direction than that of the sample to minimise the dispersion of the analyte through the minicolumn, and the eluate was taken directly to the nebulizer-burner system of the flame atomic absorption spectrometer. The minicolumn was always regenerated in the elution step using an elution time of 45 s. Signals were measured as peak area and peak height by using instrument software.

The on-line preconcentration system was optimized by using the univariate method in order to determine best chemical and flow conditions for copper(II) determination. A volume of 10 ml of work standard solution containing 50 $\mu g\,l^{-1}$ of copper was continuously injected into the flow system shown in Fig. 1, and all assays were performed in triplicate. The following buffer solutions were used for adjust the solution pH: acetic acid–cetate for the pH range of 2–4, Sörensen (Na_2HPO_4–H_2PO_4) for pH 5–7 and tris(hydroxymethyl)aminomethane $(HOCH_2)_3CNH_2$ for the pH interval of 8–10. For pH 11 only 1 $mol\,l^{-1}$ NaOH solution was utilized. A buffer volume of 5 ml was added to the working solutions containing the analyte, and the pH was adjusted with 0.01 $mol\,l^{-1}$ HNO_3 or NaOH solution. The final volume of the solution was 100 ml.

2.5. Procedures for obtaining calibration graphs without preconcentration

In this study four procedures for obtaining the calibration graphs without preconcentration were investigated. Those calibration graphs were run according to the procedures described below:

1. The on-line preconcentration system was utilized, in which the minicolumn was replaced by a manual commutator injector with a sampling loop. In this first test, two sampling loops with different internal diameters were investigated:
   (a) the first sampling loop was constructed with a polyethylene tube with 0.8 mm internal diameter, and approximately 800 mm length;
   (b) the second loop was made from a Tygon® tube, with approximately 3 mm internal diameter and 90 mm length. A polyethylene tube was connected to the extremities (i.d. = 0.8 mm with around 40 mm length). The connection between the Tygon® tube and the polyethylene tube was made with a piece of Tygon® tubing of smaller diameter.
2. Direct aspiration of 415 µl of concentrated analyte solution, contained in a small glass flask, to the nebulizer-burner system of the spectrometer was carried out.
3. Four hundred and fifteen microliters of the concentrated solution were injected through a micropipette to a small Teflon cup, connected to a Teflon tube which aspirates the sample to the nebulizer system of the spectrometer.

To obtain the calibration graph without preconcentration using the on-line system, the sampling loop was filled with a concentrated solution of the analyte. At this stage, the V_1

valve was activated during 15 s, in order to insure total filling of the sampling loop. The concentrated solution excess was discarded. The volume of this sampling loop (415 μl) was calculated taking into consideration the elution flow rate (5 ml min^{-1}) and the time (approximately 5 s) for which the transient signal was produced by the on-line preconcentration system shown in Fig. 1. To introduce the concentrated solution into the nebulizer-burner of the spectrometer, the V_1 valve was turned off and the injector was switched to the elution position. The $V_{2–4}$ valves were then activated and a nitric acid solution at the same concentration optimized by the proposed on-line preconcentration system, was pumped through the sampling loop, carrying the concentrated analyte solution to the flame.

2.6. Procedure for calculate true recovery of the analyte

True recovery of the analyte by the on-line preconcentration system, shown in Fig. 1, was investigated. To obtain the true recovery, two calibration graphs were constructed, with and without preconcentration. The graph with preconcentration was obtained, utilizing the optimized variables of the on-line preconcentration system and a preconcentration time of 90 s. After the preconcentration stage in the on-line system (Fig. 1A), the analyte was eluted off-line with HNO_3 for 15 s, which was the time used for the measurement of the analytical signal during the on-line elution step (Fig. 1A). The eluate was collected in a small glass flask, and later aspirated by the nebulizer system of the spectrometer. In order to construct a calibration graph without preconcentration, the concentrated analyte solution was introduced into small glass flasks, using a micropipette. It was then aspirated by the nebulizer system of the spectrometer. Signals were carried out as peak area.

For all the procedures of this item, it was necessary to monitor the volume of the sampling loop, the volume of the preconcentrated solution, the volume of the eluate and the calibration of the volumetric flasks and of the micropipettes, in order to minimize systematic errors. The volumes were monitored with an analytical scale, using deionized water for the calibration and volume calculations. All measurements were carried out in triplicate. The calibration graphs, with and without preconcentration, were constructed utilizing the same analyte mass, that is, the copper mass in the preconcentration solution volume was the same as that in the concentrated solution volume without the enrichment stage.

2.7. Sample treatment

The matrices analyzed were potable water, water from Peri Lake and water from Conceição Lake, Florianópolis—Brazil. The water from Conceição Lake is a saline matrix. The water samples were filtered before analysis, through a cellulose membrane (Millipore) of 0.45 μm pore size. An aliquot of water sample and 5 ml of Tris buffer solution were transferred into a 100 ml calibrated flask. The pH of the solution was adjusted to 10, with 1 mol l^{-1} NaOH solution, and this solution was diluted up to required volume with the matrix itself.

The following certified material was analysed: oyster tissue—Standard Reference Material 1566a of the NIST (National Institute of Standards and Technology, USA). For their decomposition, approximately 0.5 g of material was treated with 6 ml of 65% (v/v) HNO_3 solution and 1 ml of 30% (v/v) H_2O_2 solution in a microwave oven (Milestone), according to the program given in the manual. After the digestion of the material, the resulting solution was diluted up to required volume into a 100 ml volumetric flask. An aliquot of 10 ml was transferred to a beaker and the pH of the solution was neutralized with 1 mol l^{-1} NaOH solution. To this beaker 5 ml of Tris buffer solution was added and the pH was adjusted to 10. The solution was made up to required volume with deionized water into a 100 ml volumetric flask.

3. Results and discussion

3.1. Optimization of chemical and flow variables

In order to investigate which would be the most adequate absorption measurement of the analytical signals, taking into consideration the sensitivity and precision of the analysis, the optimization was carried out separately for peak area and peak height. The first variable optimized was the pH of the sample solution, and a pH range of 2–11 was studied. According to the results shown in Fig. 2, the maximum retention of copper(II) by the active sites of the adsorbent material occurred at pH 7 and 10, when the absorption measurement was carried out as peak area. A pH of 10 was selected for subsequent studies, since at this pH the sensitivity was slightly greater. For the measurement of the analytical signal as peak height, the maximum retention for copper(II) occurred in the pH interval of 8–10. A pH of 10 was also selected for subsequent studies, since this pH gave a lower R.S.D. value, that is, 1.4%.

The influence of the sample loading flow rate on the preconcentration of copper(II) was studied in order examine whether the interaction of the analyte with 130 mg of silica modified was fast enough for its on-line collection. A volume of 10 ml of a solution containing the analyte was injected into the system at flow rates of 2.5–8.5 ml min^{-1}. The analytical signals did not vary up to flow rate of 7.5 ml min^{-1}, for both absorption measurement, indicating that the copper(II) sorption kinetic is very rapid. This fact is a very useful feature of the proposed system, because it permits a higher sample throughout. Therefore, the flow rate of 7.5 ml min^{-1} was found to be suitable for optimum loading of the analyte and was used for further studies. The sensitivity for peak area was approximately 2.5 times greater than that of peak height. The precision of the method was good for this flow rate. The R.S.D. values were 1.0 and 1.4% for peak area and peak height, respectively.

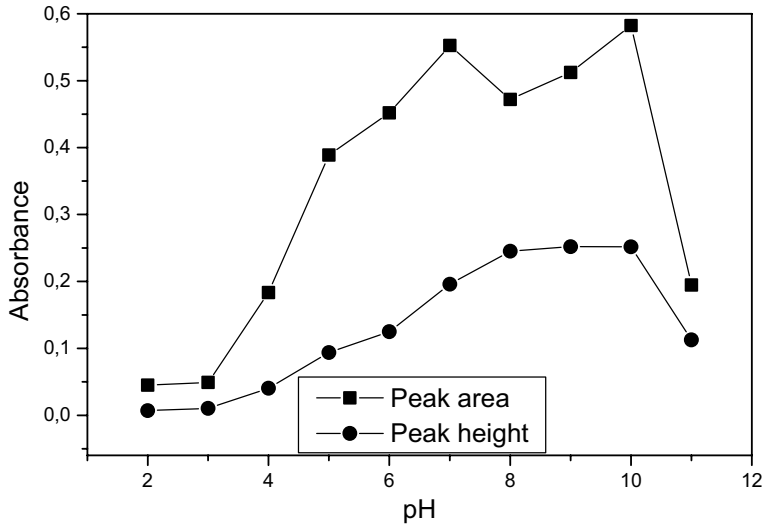

Fig. 2. Effect of pH on the preconcentration of 50 μg l^{-1} of copper(II). Sorbent: 0.13 g; 10 ml of Cu^{2+} solution were preconcentrated at flow rate of 5 ml min^{-1}. The analyte was eluted with 0.5 mol l^{-1} HNO_3 at a flow rate of 5 ml min^{-1}.

The desorption of the analyte from the minicolumn was studied using nitric acid solutions at concentrations of 0.05–3 mol l^{-1}, as stripping agent. The maximum response was obtained in the range of 0.1–2 mol l^{-1} for peak area and 0.5–2 mol l^{-1} for peak height. The concentrations selected for copper(II) elution were 0.5 and 1 mol l^{-1}, for peak area and peak height, respectively, and their respective R.S.D. values were less than 1%.

The effect of the eluent flow rate on copper desorption from the minicolumn was investigated. The effect of the nitric acid solution flow rate, on the analytical signal for copper(II), was more pronounced for peak area. The analytical signal decreased for greater flow rates, when the absorption measurement was carried out as peak area. The difference between analytical signals for peak height was insignificant in the studied flow rate range except for a flow rate of 4 ml min^{-1}, when the analytical signal was lesser. For flow rates below than 5 ml min^{-1}, there was a broadening and deformation of the transient signal, due to an incompatibility of the elution flow rate with the aspiration flow rate of the spectrometer nebulizer. Thus, a flow rate of 5 ml min^{-1} was selected for both absorption measurements and was used in subsequent experiments.

The capacity of the minicolumn packed with 130 mg of silica gel organofunctionalized was studied by measuring the maximum quantity of copper(II) sorbed on silica surface. A standard solution of 100 mg l^{-1} copper(II) was passed through of the minicolumn for 2 h at 2.0 ml min^{-1} sample flow rate. After sample loading, the minicolumn was washed with 50 ml deionized water and the analyte sorbed was eluted with 20 ml of 2 mol l^{-1} HNO_3 solution. After appropriate dilution of the eluate, the metal was measured by FAAS. The obtained maximum sorption capacity of silica gel organofunctionalized was 0.26 mmol g^{-1} for copper(II).

3.2. Interference studies

The influence of foreign ions that might be adsorbed on silica gel modified with 3(1-imidazolyl)propyl was investigated in order to identify potential interferences. The effect of each species was considered interference when the signal in the presence of the species resulted in deviation of the peak area measurement more than 5%. Optimized parameters for the preconcentration system and water samples containing 50 μg l^{-1} of copper(II) were used. The proposed procedure was applied using a solution volume of 11.2 ml (90 s of preconcentration time), and the maximum concentration of the foreign ions studied was 100 mg l^{-1}. The analyses were carried out in triplicate and the results showed that K^+, Na^+ and Ca^{2+} did not interfere. Others ions showed interference by FAAS after preconcentration using proposed method. However, these interference for the various elements occurred only at concentrations higher than those given in brackets: Al^{3+} (50 mg l^{-1}), Cd^{2+} (50 mg l^{-1}), Fe^{3+} (20 mg l^{-1}), Mg^{2+} (10 mg l^{-1}), Zn^{2+} (2 mg l^{-1}), Co^{2+} (2 mg l^{-1}) and Hg^{2+} (2 mg l^{-1}).

3.3. Study of ionic force in the on-line preconcentration process

The influence of NaCl concentration on the preconcentration of a 50 μg l^{-1} copper(II) solution was investigated. In this study, the optimized parameters and a preconcentration time of 90 s were used. The analyses were carried out in triplicate and the measurements were carried out as peak area and peak height. The NaCl concentration range studied was 0.01–5% (m/v). The results shown in Fig. 3 represent a concentration of NaCl up to 2%, in order to provide evidence that the analytical signal was greater for a 0.01% concentration of NaCl, which is the second point. The first

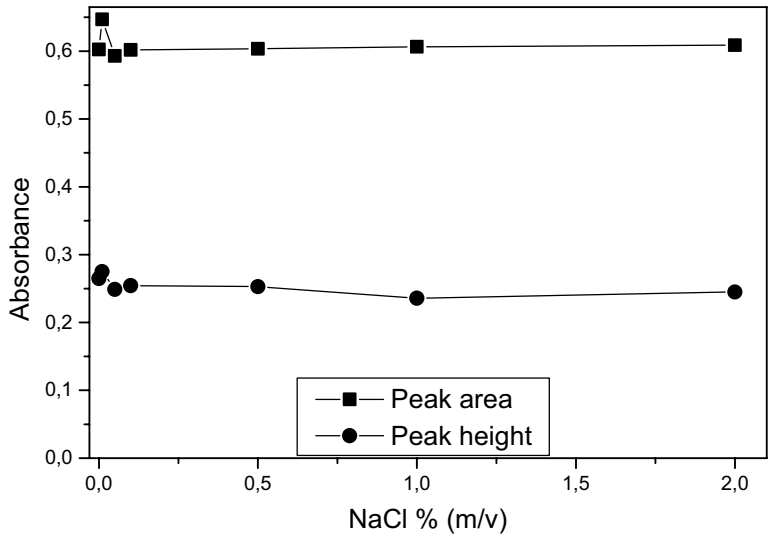

Fig. 3. Effect of NaCl concentration on the preconcentration of Cu(II) 50 μg l^{-1}. Sorbent: 0.13 g; 11.2 ml of Cu^{2+} (pH = 10) solution were preconcentrated at a flow rate of 7.5 ml min^{-1}. The analyte was eluted with 0.5 and 1.0 mol l^{-1} HNO_3 solution for peak area and peak height, respectively, with a flow rate of 5 ml min^{-1}.

point given in figure is without addition of electrolyte. There was no significant difference between the analytical signals except for the NaCl concentration of 0.01%, in the concentration range shown in Fig. 3 for both absorption measurements. The decrease in the analytical signal in relation to the first point on the graph, was greater that 10% only for peak height, for a concentration of NaCl greater than or equal to 3%. According to the results given in Fig. 3, it is possible to state that the process of copper(II) ion adsorption by the organofunctionlized silica sites, was influenced by the ionic force of the solution.

3.4. Analytical properties of merit

The analytical properties of merit were obtained using the parameters previously optimized and a preconcentration time of 90 s. The on-line preconcentration system depicted in Fig. 1 provided linear calibration graphs within the concentration range from 5 to 150 μg l^{-1} of copper(II), for both peak area and peak height. The calibration graphs were also obtained with addition of NaCl to the preconcentration solutions, so that the salt concentration of the solutions was 0.01%, since for this concentration, as shown in Fig. 3, the analytical signal was greater. The assays were performed in triplicate and the results of this study are given in Table 1.

On analyzing the slopes given in Table 1, it can be seen that the addition of NaCl to the preconcentration solutions, increased the sensitivity to 20.7 and 23.4% for peak area and peak height, respectively. Also, the calibration graph sensitivity for peak area, is approximately 2.5 times greater than that for peak height. The addition of NaCl, along with increasing the sensitivity, also provided a better linear correlation coefficient (R) for both absorption measurements, peak area and peak height. It is probable that the efficiency of the minicolumn containing modified silica is related with the ionic force of the preconcentration solution. Thus, the same concentration of NaCl given above was used in the preconcentration solutions for subsequent experiments.

The precision of the proposed method was calculated as the R.S.D. for a solution containing 20 μg l^{-1} of copper(II), when 11.2 ml of the solution was preconcentrated ($n = 7$). The R.S.D. values obtained were 1.2 and 1.4% for peak area and peak height, respectively. The detection limit was defined as the analyte concentration which resulted in a response equivalent to three times the standard deviation (S.D.) of the blank ($n = 11$), using a preconcentration time of 90 s. The values obtained were 0.4 and 0.2 μg l^{-1} of Cu(II) for peak area and peak height, respectively. The sampling frequency of the procedure was 27 samples per hour, for a 90 s preconcentration time.

3.4.1. Calculation of the enrichment factor

Four procedures for obtaining the calibration graphs without preconcentration were investigated, since some studies in the literature do not give clear evidence as to how the calibration graph without preconcentration, is constructed. The enrichment factors were calculated as the ratio of the

Table 1
Analytical data obtained from the equations of the calibration graphs with and without addition of NaCl to the preconcentration solutions

Absorbance	NaCl added (μl)[a]	Range (μg l^{-1})	Slope	R[b]
Peak area	–	5–150	0.00876	0.9934
	100	5–150	0.01057	0.9972
Peak height	–	5–150	0.00337	0.9894
	100	5–150	0.00416	0.9936

[a] Addition of 100 μl of 10% (m/v) NaCl in 100 ml of solution.
[b] Linear correlation coefficient.

Table 2
Values obtained for the enrichment and recovery factors using the proposed method, according to the procedure for obtention of calibration graph construction without preconcentration

Absorbance	A[a]	B[b]	R[c]	EF[d]	RF (%)[e]
Peak area	1a	0.000758	0.99981	19.5	75.8
	1b	0.000714	0.99998	20.7	80.5
	2	0.000572	0.99963	25.8	10.3
	3	0.000582	0.99963	25.4	98.8
Peak height	1a	0.000136	0.99998	39.4	153.3
	1b	0.000127	0.99998	42.2	164.1
	2	0.000148	0.99987	36.2	140.8
	3	0.000147	0.99955	36.5	141.9

[a] Procedures for obtention of the calibration graphs without preconcentration.
[b] Slope.
[c] Linear correlation coefficient.
[d] Enrichment factor.
[e] Recovery factor.

slopes of the calibration graphs obtained with and without preconcentration. All the statistical calculations were based on the average of triplicate readings for each standard solution. The calibration graphs with preconcentration using the optimized parameters, five standard solutions of concentrations 10, 20, 30, 40 and 50 $\mu g\,l^{-1}$ of Cu(II) and a preconcentration time of 90 s, resulted in the following equations:

$A = 0.05121 + 0.01479C$ ($r = 0.99959$) for peak area,

$A = 0.01984 + 0.00536C$ ($r = 0.99936$) for peak height,

where A is absorbance and C is concentration in $\mu g\,l^{-1}$.

The calibration graphs without preconcentration were obtained according to the four procedures described in Section 2.5. The concentration range of the concentrated analyte solution was of 81.25–406.25 $\mu g\,l^{-1}$, and the results of this study are given in Table 2.

In order to discuss the results of Table 2, the true recovery of the analyte by the on-line preconcentration system, was investigated. To obtain the true recovery, two calibration graphs were constructed, with and without preconcentration, according to the procedure described in Section 2.6. The two graphs obtained, with and without preconcentration, gave the following equations, respectively:

$A = 0.01901 + 0.00812C$, for the interval of 10–50 $\mu g\,l^{-1}$ ($r = 0.99965$),

$A = 0.00575 + 0.00121C$, for the interval of 81.25–406.25 $\mu g\,l^{-1}$ ($r = 0.99997$).

The recovery was then found by the following equation:

$$EF = RF\left(\frac{V_S}{V_E}\right),$$

where EF is the enrichment factor, RF the recovery factor, V_S the volume of preconcentrated sample and V_E the volume of eluate.

Obtaining EF by the slopes of the calibration graphs with and without preconcentration, it was then possible to find RF, considered as the true recovery of analyte by the on-line preconcentration system. The value found was 82.6%.

On analyzing the results in Table 2, it could be stated that procedure 1b, which utilizes the on-line preconcentration system with a sampling loop and the measurements of the absorption signal as peak area, is the most appropriate mode to calculate the enrichment factor, since the recovery obtained with this procedure was 80.5%, a difference of only 2.1% from the true recovery. In procedure 1a, using the measurement as peak area, the recovery was lesser, that is, 75.8%. This procedure had previously been proposed by our research group [30]. However, in this study, it was observed that the internal diameter of the sampling loop in the on-line system, has some influence on the enrichment factor calculation. Thus, an internal diameter of 3 mm for the sampling loop is more adequate and also corresponds to the internal diameter of the minicolumn of the on-line preconcentration system. The direct aspiration of the concentrate to the nebulizer system of the spectrometer, according to procedures 2 and 3, gave a greater enrichment factor, when the measurement was carried out as peak area. However, there is a discrepancy of almost 20% between the recovery factors found with these procedures (100.3 and 98.8%) and the true analyte recovery (82.6%).

The true analyte recovery by the on-line preconcentration system shown in Fig. 1, was also investigated for peak height, according to the procedure described for peak area. The true analyte recovery was 79.1% which is very close to the value found for peak area (82.6%). The values for the recovery factors, given in Table 2 for peak height, showed a great discrepancy when compared to the true recovery value. This indicates the difficulty in producing the same transient signal generated by the on-line preconcentration system, which presents a higher peak due to the concentration gradient provided by the minicolumn. Thus, the measurement of the analytical signal as peak height, allows the achievement of higher values for the enrichment and recovery factors.

The obtained enrichment factors by the proposed on-line preconcentration system were thus comparable to those obtained by other methods described in the literature [18,30–36]. Highers enrichment factors can be achieved by using sample volumes greater than 11.2 ml (>90 s preconcentration time).

3.5. Application of the proposed method

The proposed procedure was applied to the determination of copper(II) in water samples. The analyte was previously enriched in the preconcentration system shown in Fig. 1, utilizing the optimized variables and a preconcentration time of 90 s. In all samples analyzed, the concentration of the analytes is close to the detection limit of the method, since the analyte was not detectable with the on-line preconcentration system. The accuracy of the method was investigated

Table 3
Copper(II) recovery for the water samples

Aqueous matrix	Cu^{2+} added ($\mu g\,l^{-1}$)	Cu^{2+} found ($\mu g\,l^{-1}$)	Recovery (%)
Absorbance: peak area			
Peri Lake	10	10.72 ± 0.34	107.2
	20	21.09 ± 0.12	105.4
	40	42.45 ± 0.09	106.1
Potable water	10	10.65 ± 0.35	106.5
	20	20.41 ± 0.18	102.0
	40	40.94 ± 0.27	102.4
Conceição Lake	10	11.21 ± 0.25	112.1
	20	21.24 ± 0.49	106.2
	40	39.21 ± 0.04	98.0
Absorbance: peak height			
Peri Lake	10	5.71 ± 0.12	57.1
	20	14.00 ± 0.54	70.0
	40	31.73 ± 0.38	79.3
Potable water	10	9.28 ± 0.08	92.8
	20	20.25 ± 0.25	101.2
	40	39.77 ± 1.08	99.4
Conceição Lake	10	5.39 ± 0.14	53.9
	20	12.62 ± 0.02	63.1
	40	27.70 ± 0.95	69.2

through the recovery test, adding known masses of analyte to the analyzed aqueous matrices. In Table 3, the results of the recovery test are given. The measurement of the analytical signals were carried out as peak area and peak height, in order to verify which would be the most adequate mode of the absorption measurement for the proposed system. Each sample was analyzed in triplicate.

As seen in Table 3, the efficiency of the on-line preconcentration system was very good for all samples analyzed, when the measurement of the analytical signal was carried out as peak area. For this type of measurement, the recoveries were between 98.0 and 112.1%, indicating that the matrix effect was not significant. The recovery values shown in Table 3, for water from Conceição Lake, that is a saline matrix, and absorbance as peak area, were obtained with a preconcentration flow rate of 4 ml min^{-1}, since the flow rate selected in the flow system, which was 7.5 ml min^{-1}, was giving recoveries of approximately 85%. Thus, the matrix effect was minimized by the decrease in sample flow rate, which allowed a greater contact time of the analyte with the adsorbent material.

For recoveries using the absorption measurement as peak height, the method was more susceptible to matrix effect, except for the potable water sample. The recoveries found, for the water samples from Conceição Lake and Peri Lake were between 53.9 and 79.3%. This more pronounced matrix effect, for both matrices analyzed, occurred due to the broadening of the transient signal. Therefore, there was a greater dispersion of retained analyte in the minicolumn, because of the matrix effect, and this resulted in a lower value for peak height. However, this effect did not adversely affect the signal as peak area. It may therefore be concluded, that the analytical signal as peak area, is the most adequate measurement for the proposed procedure.

Table 4
Determination of copper(II) in a certified material

Sample flow rate (ml min^{-1})	Certified values ($\mu g\,g^{-1}$)	Found values ($\mu g\,g^{-1}$)	Recovery (%)
7.5	66.3 ± 4.3	59.83 ± 0.40	90.2
4.0	66.3 ± 4.3	63.14 ± 0.54	95.2

3.6. Analysis of certified material

The accuracy of the on-line preconcentration system was also investigated for the determination of copper(II) in oyster tissue—Standard Reference Material 1566a of the NIST. The analyses were carried out in triplicate and the analytical signal measurement was taken as peak area. The copper(II) determination for the certified sample was carried out using the optimized variables of the on-line system and a preconcentration time of 90 s. According to Table 4, the analyte recovery for the certified material was 90.2% when a preconcentration flow rate of 7.5 ml min^{-1} was employed. In order to achieve a greater recovery, the preconcentration was carried out with a flow rate of 4 ml min^{-1} and a preconcentration time of 120 s. For this new flow rate, a recovery of 95.2% was obtained, revealing that the proposed method showed good accuracy for the copper(II) determination by the on-line preconcentration system.

4. Conclusions

The silica organofunctionalized with 3(1-imidazolyl)propyl, contained in a minicolumn of the proposed on-line preconcentration system presented relatively high adsorption and desorption kinetics, thereby allowing the achievement of good sensitivity and high analytical velocity. For the analytical signal measurement as peak area, the method gave a good accuracy for the analysis of the certified material, and also for the recovery test applied to the aqueous samples. The absorbance measurement as peak height was more subject to matrix interference, however, this type of reading allowed the achievement of higher enrichment factors. The values for the enrichment factors, obtained in this study, are closely related to the procedures adopted for the construction of the calibration graph without preconcentration. The flow analysis mode offered simplicity, flexibility and good precision.

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