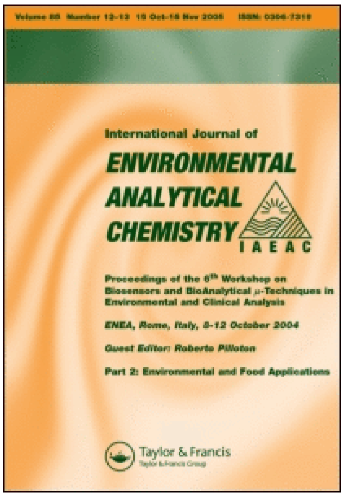

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Factorial design for optimization of flow-injection preconcentration procedure for copper(II) determination in natural waters, using 2-aminomethylpyridine grafted silica gel as adsorbent and spectrophotometric detection

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The 2-aminomethylpyridine anchored silica gel (AMPSG) was successfully used as a sorbent in a simple spectrophotometric flow system for Cu^{2+} preconcentration in natural water samples, using sodium diethyldithiocarbamate as chromogenic agent (460 nm). The system was optimized using a full factorial design 2^5 to determine better analytical conditions to determine copper in the natural water samples such as those from river, tap, stream, spring, well, waste, synthetic brackish water and a water reference material (NIST-1640). The better conditions used were: 180 s loading; 30 s elution; 30 s regeneration of the column; loading flow rate 6.6 mL min^{-1}; buffer solution for the preconcentration and regeneration of the column-acetate buffer pH 5.75; elution flow rate 1.6 mL min^{-1}; eluent composition 0.20 mol L^{-1} HNO_3. Under these conditions, the preconcentration factor obtained was 77, and the detection limit achieved was 3.0 ng mL^{-1}. The recovery of spiked water samples ranged from 95.2 to 104.7%.

Keywords: Copper preconcentration; 2-Aminomethylpyridine grafted silica gel; Flow injection; Spectrophotometry; Factorial design; Water samples

1. Introduction

The determination of potentially toxic elements in environmental samples has been a common concern everywhere. Usually, these element levels found in environmental samples are very low, requiring the use of high-detection-power techniques such as graphite furnace atomic absorption spectrometry (GFAAS) [1–4] and inductively coupled plasma mass spectrometry (ICP-MS) [5, 6]. In underdeveloped countries, these analytical techniques are not extensively used due to their high implementation

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and maintenance costs [1–6]. An interesting alternative for the determination of low levels of potentially toxic elements in environmental samples is the use of a preconcentration procedure. Among several kinds of preconcentration methods, solid-phase extraction using chelating sorbents [7–13] and also ion exchangers [14–16] is the most valuable method, since it does not require the use of toxic organic solvents, which generates large volumes of waste that need to be treated subsequently, besides being a time-consuming procedure that leads to a decrease in sampling throughput [15–17].

Modified silica with different organic groups is one of the most successful sorbents used in the analytical laboratories, because the silica supports do not swell or shrink like polymeric resins [14] and unmodified natural occurrence materials [18]; this means that the modified silica sorbent can be used for several cycles of preconcentration, because retention processes such as adsorption, chelation, and ion exchange are reversible [13, 19]. The modified silica is useful to employ in aqueous and organic solvents media [13, 19] due to the good thermal stability [13, 19] and appropriate accessibility of ions to the attached chelating groups, which allows its high preconcentration factors [13, 19].

Flow-injection preconcentration systems using minicolumns packed with a suitable sorbent for the preconcentration of several analytes in many different kinds of sample have been used successfully [15–17]. These procedures present several advantages over conventional column-preconcentration procedures. For example, the total time of preconcentration can be reduced from several hours to carry out just one cycle [15, 16] to just a few minutes, thus increasing the sampling throughput remarkably; there is a higher repeatability of measurements acquired with flow systems [17]; the contamination risk is reduced because there are fewer manual operations [15–17]; and there is a lower consumption of samples and reagents [15–17]. Flow-injection preconcentration systems present several advantages when coupled mainly with flame atomic absorption spectrometry (FAAS) [20–32] and inductively coupled plasma atomic emission spectrometry (ICP-AES) [32–37]. On, the other hand, the applications in which flow-injection preconcentration systems are coupled to spectrophotometry for the determination of metallic ions are rare [38, 39]. Also, the use of organofunctionalized silica gel used as a sorbent in flow systems for Cu^{2+} determination has also rarely been used [25, 39], so there is a need to explore these more extensively.

The performance of a flow-preconcentration system requires the optimization of several variables. Conventionally in the optimization of the system, each parameter is varied at the time, and the others are fixed. By using the conventional univariate mode, it is not possible to evaluate the interactions among different variables [40], which limit the overall optimization of the system [33, 40, 41]. In order to overcome these difficulties, a factorial design can be used, where all the variables are changed simultaneously [40], and the maximum optimization of the system can be achieved with a minimum number of experiments [33, 40, 41].

In this investigation, the sorbent 2-aminomethylpyridine grafted silica gel (AMPSG) was successfully used for the preconcentration of copper (II) in natural water samples. A flow system for online preconcentration of the analyte was used with a spectrophotometric detection at 460 nm, by using sodium diethyldithiocarbamate (DDTC) as a chromogenic agent. The system was optimized, employing a full factorial design 2^5 (32 experiments) in order to find the best analytical conditions for copper preconcentration and reducing the total number of the experiments to attain this goal.

2. Experimental

2.1 *Instruments*

A 600 S Femto spectrophotometer (São Paulo-SP, Brazil) provided with a 150 µL flow cell (Femto) and a serial port RS232C connected to an AMD K6II 350 MHz personal computer for data acquisition were used throughout for the analytical measurements. Two four-channel Milam bp-200 peristaltic pumps (Colombo-PR, Brazil) provided with Tygon® and silicone tubes of different diameters were used for the propulsion of solutions in the flow system. For the pH measurements, a handylab 1 Schott hand-held pH/mV meter (Mainz, Germany) was used with a combined glass electrode model Blue-Line 23 pH.

2.2 *Reagents, solutions, and samples*

Double-distilled water was used throughout. Solutions containing 0.10–1.0 mol L^{-1} of nitric acid (Merck, Rio de Janeiro-RJ, Brazil) were used as eluent. A 1.00 g L^{-1} copper (II) stock solution was prepared, dissolving 0.5000 g of metallic copper (Merck, Rio de Janeiro-RJ, Brazil) in 10 mL of 7.0 mol L^{-1} of nitric acid; this solution was quantitatively transferred to a 500.0 mL calibrated flask, and the volume was made up to the mark with water. The calibration solutions within 50–300 ng mL^{-1} (preconcentration) and 2000–8000 ng mL^{-1} (without preconcentration) of the Cu^{2+} range were prepared by suitable serial dilution of the stock solution with water and adjusting the final acidity to pH 1.0 with HNO_3.

Sodium diethyldithiocarbamate (DDTC; Merck, Darmstadt-Germany) 8.7×10^{-3} mol L^{-1} solution was prepared daily by dissolving 0.375 g of DDTC in 5 mL of ethanol; afterwards, this solution was mixed with 150 mL of hot water (70–80°C), filtered into a 250.0 mL volumetric flask, and the volume made up to the mark with water.

For the buffer preparations, glacial acetic acid (Merck, Rio de Janeiro, Brazil), sodium acetate (Merck), ammonium acetate (Merck), boric acid (Merck), sodium tetraborate decahydrate (Vetec), potassium hydroxide (Reagen), and nitric acid (Merck) were used.

For the interference studies and synthetic water samples, the following salts of elements were used: NaCl (Merck), KCl (Merck), $MgSO_4 \cdot 7H_2O$ (Merck), $CaSO_4 \cdot 2H_2O$ (Merck), $AlCl_3 \cdot 6H_2O$ (Merck), $CoSO_4 \cdot 7H_2O$ (Vetec), $Ba(NO_3)_2$ (Reagen), $CrCl_3 \cdot 6H_2O$ (Vetec), $FeSO_4 \cdot NH_4SO_4 \cdot 6H_2O$ (Merck), $MnSO_4 \cdot H_2O$ (Reagen), $ZnSO_4 \cdot 7H_2O$ (Nuclear), and $NiSO_4 \cdot 6H_2O$ (Reagen).

The 2-aminomethylpyridine anchored silica gel (AMPSG) sorbent was prepared as reported elsewhere [42], obtaining a material with a specific surface area of $121.4 \pm 8.6\,m^2\,g^{-1}$ and a coverage of silica surface of 0.76 ± 0.04 mmol of 2-aminomethylpyridine groups per gram of sorbent.

The ordinary water samples (river, stream, spring, tap, waste, well) were filtered with Whatman paper, and the pH was adjusted to 1.0 with HNO_3. The samples were then analysed using the procedure of preconcentration. The reference material, Trace Elements in Natural Water (NIST-1640), from the National Institute of Standards and Technology (NIST, Gaithersburg, USA) was used to achieve the required accuracy of the method.

2.3 *Flow system*

For Cu^{2+} determination in the water samples, a manually operated manifold made of a Perspex proportional injector-commutator [43] containing a 2 : 3 : 2 section for adapting the sampling loop and/or preconcentration column and a 0 : 1 : 2 section for delivering the chromogenic agent intermittently were used. The central part of the injector-commutator was movable and the edge portions were fixed. The PVC mini-column was placed in the central position of the injector-commutator and was used for the analyte preconcentration. Also, two peristaltic pumps, 0.8 mm ID polyethylene transmission lines, Perspex connectors, flow cell and spectrophotometer were mounted as depicted in figure 1. In the preconcentration position (position I, figure 1A), the

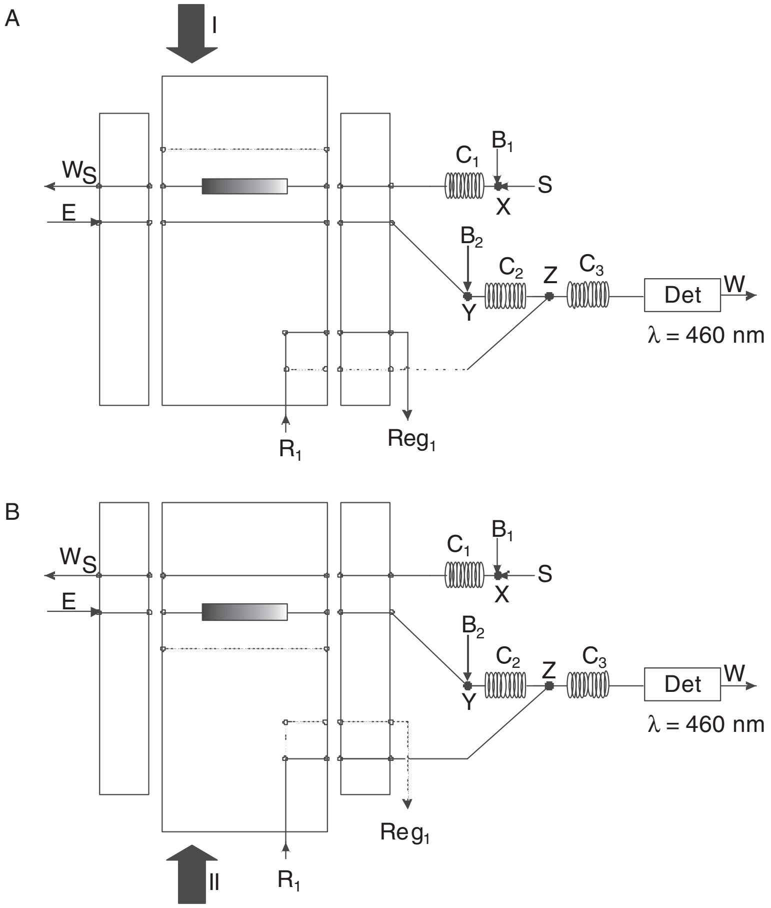


Figure 1. Manifold employed for the determination of Cu^{2+} in water samples. (A) Sampling position using the preconcentration system. (B) Eluting position of the preconcentration system. B_1: $2.0\,mol\,L^{-1}$ acetate buffer solution for the preconcentration (pH 5.75). $4.5\,mL\,min^{-1}$; S: sample solution (pH 1), $5.0\,mL\,min^{-1}$; C_1: coil reactor (100 μL) for mixing sample solution and buffer B_1; W_S: waste of sample solution and buffer B_1; E: eluent $0.60\,mol\,L^{-1}$ HNO_3. $2.6\,mL\,min^{-1}$; R_1 $8.7 \times 10^{-3}\,mol\,L^{-1}$ DDTC. $2.2\,mL\,min^{-1}$; Reg_1: regeneration vessel of DDTC; B_2: $2.0\,mol\,L^{-1}$ acetate buffer solution (pH 5.75) for colour-forming reaction. $2.6\,mL\,min^{-1}$; C_2: coil reactor (100 μL) for buffering the sample zone; C_3: coil reactor (200 μL) for colour developing; Det: spectrophotometric flow cell. 150 μL. $\lambda = 460\,nm$. X, Y, Z: confluence points.

sample solution and the buffer solution B_1 (2.0 mol L^{-1} acetate buffer, pH 5.75) were merged in the confluence point X; then, these solutions were mixed in the coil C_1 (100 μL) and directed to the minicolumn, for sorbing the analyte present in the sample solution, and the aqueous solution was directed to the waste W_S. The chromogenic reagent (8.7×10^{-3} mol L^{-1} DDTC) R_1 was pumped to the regeneration vessel Reg_1. In the second channel, the carrier solution E (HNO_3) was pumped to the system where, at confluence point Y, it merged with B_2 buffer solution (2.0 mol L^{-1} acetate buffer, pH 5.75) and mixed in the coil mixer C_2 (200 μL). Then, the buffered sample zone reached the confluence point Z and subsequently reached the mixture coil C_3 (200 μL); this sampling zone passed along the flow-cell (150 μL) of the spectrophotometer (460 nm) producing the baseline, and subsequently this solution was directed to the waste (W). After the preconcentration period, the central part of the injector-commutator was slid to the position II (figure 1B), and the minicolumn was inserted in the eluent line, eluting the retained analyte. At the confluence point Y, the sample zone merged with B_2 buffer solution and mixed in the coil mixer C_2; then, the buffered sample zone reached the confluence point Z where it merged with the chromogenic reagent and then mixed in the coil mixer C_3 (200 μL) forming the $Cu(DDTC)_2$ complex [44]. This zone was subsequently directed to the flow cell of the spectrophotometer (460 nm), producing a transient signal, and finally this zone was directed to the waste (W).

2.4 *Full factorial design*

One of the simplest types of factorial designs used in chemistry is one having two levels. The design determines which factors have important effects on a response as well as how the effect of one factor varies with the level of the other factors. Effects are differential quantities expressing how a response changes as the levels of one or more factors are changed. The determination of factor interactions can be important for successful system optimization. In this investigation of copper preconcentration, the analytical signal could depend on mass of the AMPSG sorbent present in the minicolumn, the time of preconcentration, eluent flow rate, analyte flow rate for the preconcentration, and composition of the eluent. Other variables such as coil volumes (C_1, C_2 and C_3) were optimized previously to the full factorial design, in order to obtain undistorted peak profiles, and also the optimum pH for preconcentration was also previously optimized in univariate mode, because it is known that at some pH values, the adsorption of any analyte does not occur; if this were used incorrectly in the full factorial design, it could lead to a misinterpretation of the results. In addition, it is important to keep some parameters constant in order to diminish the total number of experiments of the full factorial design.

The effects of these factors and their interactions were measured by performing 32 experiments forming the 2^5 full factorial designs given in table 1. For convenience, each factor level was coded as −1 (low) or +1 (high), and the effect values were calculated using the following equation [27, 28]:

$$Ef_i = R_i^{(+)} - R_i^{(-)},$$

Table 1. Optimization of flow system for Cu^{2+} preconcentration using AMPSG as sorbent.[a]

Experiment	A	B	C	D	E	Analytical signal/10^{-3}
1	−	−	−	−	−	38.7
2	+	−	−	−	−	64.0
3	−	+	−	−	−	205.7
4	+	+	−	−	−	182.0
5	−	−	+	−	−	34.0
6	+	−	+	−	−	61.3
7	−	+	+	−	−	181.3
8	+	+	+	−	−	183.0
9	−	−	−	+	−	141.3
10	+	−	−	+	−	142.3
11	−	+	−	+	−	453.3
12	+	+	−	+	−	420.7
13	−	−	+	+	−	158.3
14	+	−	+	+	−	115.7
15	−	+	+	+	−	421.7
16	+	+	+	+	−	422.3
17	−	−	−	−	+	46.7
18	+	−	−	−	+	28.3
19	−	+	−	−	+	251.3
20	+	+	−	−	+	182.0
21	−	−	+	−	+	37.7
22	+	−	+	−	+	66.3
23	−	+	+	−	+	196.7
24	+	+	+	−	+	305.7
25	−	−	−	+	+	270.7
26	+	−	−	+	+	134.3
27	−	+	−	+	+	468.0
28	+	+	−	+	+	441.0
29	−	−	+	+	+	145.0
30	+	−	+	+	+	132.7
31	−	+	+	+	+	436.7
32	+	+	+	+	+	447.7

		Levels	
Variables		−	+
A	Column mass (g)	0.1002	0.1512
B	Preconcentration time (s)	60	180
C	Eluent flow rate ($mL\,min^{-1}$)	1.6	4.0
D	Cu^{2+} flow rate ($mL\,min^{-1}$)	2.5	6.6
E	Eluent concentration (HNO_3; $mol\,L^{-1}$)	0.2	0.6

[a] Factorial design 2^5 (32 experiments) aiming the maximum analytical signal. Fixed conditions: 100 ng mL^{-1} Cu^{2+} (pH 1); 2.0 mol L^{-1} acetate buffer pH 6 (buffer B_1 and B_2); chromogenic agent 8.7×10^{-3} mol L^{-1} DDTC, 2.2 mL min^{-1}.

where $R_i^{(+)}$ and $R_i^{(-)}$ are the averages of results when the *i*th factor is at its high (+) or low (−) level independent of the signs of the other effects. Sixteen experimental values make up each average because each column in table 1 has 16 positive and 16 negative signs. The 32 experiments were performed in random order. This complete factorial design is particularly efficient for the evaluation of the main effects of each factor and of the interactions of two or more factors on the Cu^{2+} preconcentration. Univariate methods are unable to measure these interactive effects [33, 40, 41]. The definitions of the factors and levels used in the full factorial design and the analytical signal achieved for Cu^{2+} preconcentration are also presented in table 1. These listed numbers represent the mean value for three independent determinations.

3. Results and discussion

3.1 *Preliminary experiments*

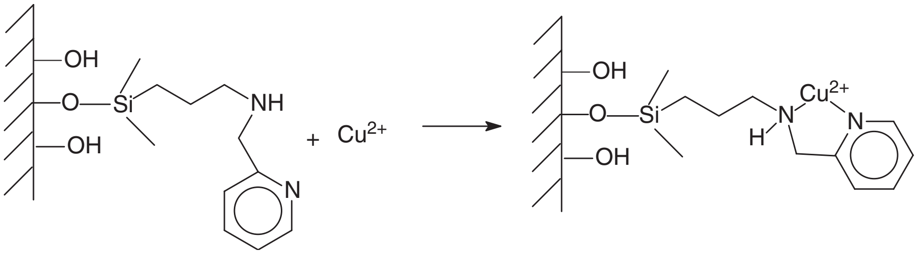
The best performance of a factorial design depends on some knowledge about the system to being optimized [40]. If the effects of all system parameters to be studied are unknown, the projection of the results obtained with the factorial design could not attain the maximum optimization of the overall system [40]; besides, the total number of experiments required will be excessively increased, making the factorial design very complex [40]. Based on this, preliminary experiments on the optimization of flow-injection preconcentration were carried out replacing the minicolumn with a sample loop of 375 µL (75 cm, 0.8 mm i.d.), and the buffer solution B_1 was not necessary in these experiments. The first variable investigated for the optimization of the reaction between Cu^{2+} and DDTC was the pH (buffer solution B_2). Several 0.8–2.0 mol L^{-1} buffer solutions, to support higher acidic concentrations of real samples, with pHs ranging from 4.5 to 10.5, were tested. The pH interval that gave a higher analytical signal ranged from 5.0 to 6.5. The 2.0 mol L^{-1} acetate buffer, with pH adjusted to 5.75, was chosen for the colour-forming reaction (buffer solution B_2). The flow rate of the B_2 buffer solution was also investigated from 2.0 to 5.0 mL min^{-1}. It was observed that the best sensitivity associated with higher reproductivity of the results occurred at flow rates of 2.5 mL min^{-1}. For flow rates higher than 3.5 mL min^{-1}, the RSD for five measurements was worse than 12%.

The concentration of DDTC was fixed at 8.7×10^{-3} mol L^{-1} because of the limited solubility of this reagent however the chromogenic agent flow rate was investigated. It was observed that DDTC flow rates ranging from 2.0 to 4.0 mL min^{-1} did not present significant changes in the analytical signals. A flow rate of 2.2 mL min^{-1} DDTC was used throughout this work. It is important to point out that the colour-forming reagent was introduced intermittently in the flow system, thus saving this reagent [45]. Considering a time of 60 s for a colour-developing reaction, a mass of 3.30 mg of DDTC was used for each determination. The introduction of the chromogenic agent intermittently was only possible because this reagent solution is colourless and does not cause any changes in the baseline [45].

The dimensions of coil reaction C_2 and C_3 were also investigated, and the dimensions that promoted the highest and sharpest analytical signal were 100 and 200 µL, respectively. The C_2 reaction coil served to buffer the sample zone prior to the addition of the chromogenic agent. After adding the DDTC to the buffered sample zone, this reaction zone was mixed in the reaction coil C_3. It should be stressed that higher volumes of reaction coils (C_2 and C_3) led to broad peak profiles that lasted more than 20 s to return to the baseline after the appearance of the maximum of the peak. The conditions optimized above were used in the flow-injection preconcentration procedure.

3.2 *Optimization of the copper flow-injection preconcentration system and figures of merit*

The AMPSG sorbent presents two chelating sites to the metal ion, as depicted in scheme 1. The formation of five- and six-member-ring chelates is thermodynamically favourable, leading to high complex formation constants (K_f); however, the conditional



Scheme 1. Sorption of Cu^{2+} on AMPSG.

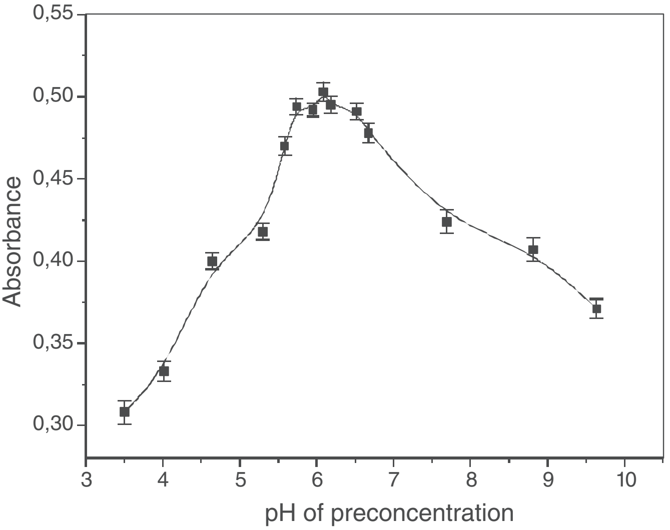


Figure 2. Effect of the acidity on the sorption of Cu^{2+} on AMPSG. Chromogenic agent 8.7×10^{-3} mol L^{-1} DDTC, 2.2 mL min^{-1}; buffer for colour-forming reaction: 2.0 mol L^{-1} acetate buffer solution (pH 5.75); 2.6 mL min^{-1}; 250 ng mL^{-1} Cu^{2+}; sample flow rate 5.0 mL min^{-1}; time of preconcentration 120 s; buffer flow rate 3.6 mL min^{-1}; eluent 0.40 mol L^{-1} HNO_3, 2.0 mL min^{-1}; time of elution 30 s; time of regeneration 30 s.

complex formation constant depends strongly on the pH of the reaction [46]. Figure 2 shows the effect of the pH, of the buffer solution in the entrance of the minicolumn, on the sorption of Cu^{2+} on the AMPSG. As can be seen, the maximum signal was obtained when the pH of the buffer solution in the entrance of the minicolumn was fixed at 5.5–6.5. Based on these results, the buffer solution used for the preconcentration of Cu^{2+} in acidic water samples (pH 1) was 2.0 mol L^{-1} acetate buffer pH adjusted to 5.75. It should be stressed that the pH measured at the exit of the minicolumn was 5.4.

Statistical methods of experimental design and systematic optimization such as factorial design have been applied to different systems [33, 40, 41], because of their abilities to extract relevant information from systems while requiring a minimum number of experiments. In this context, a full factorial design (2^5, $n = 32$ experiments) was carried out [33, 40, 41] to optimize the flow system for Cu^{2+} preconcentration using AMPSG as sorbent. The aim of this factorial design was to obtain the maximum analytical signal, which means a high preconcentration of the system allowing the

Cu^{2+} determination in low concentrations. The definitions of the factors and levels used in the complete design are presented in table 1.

The estimated effects and the coefficients of the model were calculated using Minitab Statistical Software Release 13.20 TM for calculating all the statistical parameters, and the results are presented in table 2. As can be seen, the variables time of preconcentration (B) and analyte flow rate (D) (sampling loading), as well as their interaction (BD), had more significant effects on the overall preconcentration system. The statistical significance of all the effects can be determined by plotting the effect values on a cumulative probability graph [40] (see figure 3A). The effect values are graphed on the abscissa, whereas the expected normal score values for a normal distribution are graphed on the ordinate. Points corresponding to a normal distribution are expected to be located on a vertical line centred about the origin. The effects these points

Table 2. Main, interaction effect, coefficients of the model, and standard deviation of each effect and coefficient for the full 2^5 factorial design.

	Effects/10^{-3}	Coefficients/10^{-3}
Average		
I	–	213.0 ± 6.2
Main effects		
a	-9.9 ± 12.3	4.93 ± 6.2
b	223.9 ± 12.3	111.9 ± 6.2
c	-7.8 ± 12.3	-3.88 ± 6.2
d	167.9 ± 12.3	83.9 ± 6.2
e	22.8 ± 12.3	11.4 ± 6.2
Interaction of two factors		
ab	6.1 ± 12.3	3.0 ± 6.2
ac	25.2 ± 12.3	12.6 ± 6.2
ad	-19.9 ± 12.3	9.9 ± 6.2
ae	-4.5 ± 12.3	-2.2 ± 6.2
bc	6.7 ± 12.3	3.3 ± 6.2
bd	60.0 ± 12.3	30.0 ± 6.2
be	9.6 ± 12.3	4.8 ± 6.2
cd	-16.2 ± 12.3	-8.1 ± 6.2
ce	1.0 ± 12.3	0.5 ± 6.2
de	2.2 ± 12.3	1.1 ± 6.2
Interaction of three factors		
abc	9.1 ± 12.3	4.5 ± 6.2
abd	11.7 ± 12.3	5.9 ± 6.2
abe	14.2 ± 12.3	7.1 ± 6.2
acd	-6.3 ± 12.3	-3.2 ± 6.2
ace	23.1 ± 12.3	11.6 ± 6.2
ade	-6.9 ± 12.3	-3.5 ± 6.2
bcd	3.6 ± 12.3	1.8 ± 6.2
bce	11.2 ± 12.3	5.6 ± 6.2
bde	-15.8 ± 12.3	-7.9 ± 6.2
cde	-15.1 ± 12.3	-7.5 ± 6.2
Interaction of four factors		
abcd	-10.3 ± 12.3	-5.1 ± 6.2
abce	-3.4 ± 12.3	-1.7 ± 6.2
acde	1.2 ± 12.3	0.6 ± 6.2
abde	-1.6 ± 12.3	-0.8 ± 6.2
bcde	4.2 ± 12.3	2.1 ± 6.2
Interaction of five factors		
abcde	-16.9 ± 12.3	-8.4 ± 6.2

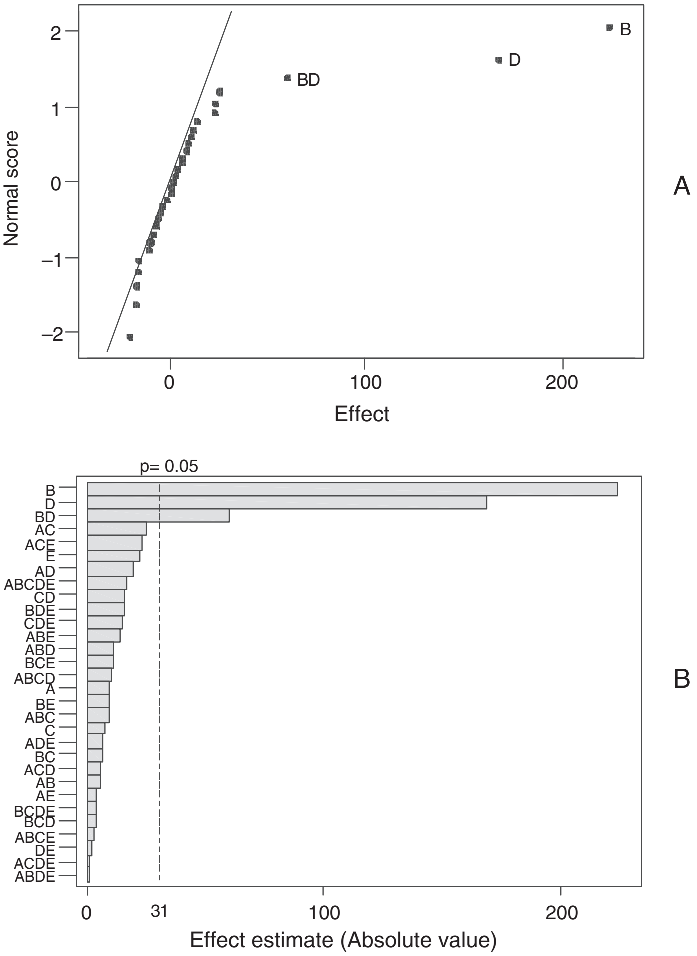


Figure 3. (A) Cumulative probability graph for the effect values of the 2^5 factorial design of table 2. (B) Pareto plot of the effects on the Cu^{2+} preconcentration, at 5% probability.

represent are not significant and only provide estimates of effect errors. Only three points are clearly removed from the line in the centre of the graph, and have statistically significant effect values: the variables time of preconcentration (*B*), analyte flow rate or sampling loading (*D*) and its interaction (*BD*). The variables mass of sorbent present in the minicolumn (*A*), eluent flow rate (*C*), and eluent composition (*E*) did not have any significant effects on the overall preconcentration. As a confirmation of these results, figure 3B shows the Pareto plot of all estimated effects. As can be seen at 5% probability, only the variables *B* and *D* and their interaction (*BD*) are significant, and all other effects could be eliminated from the model. Therefore, the full 2^5 factorial design (32 experiments) could be reduced to a full 2^2 factorial design with eight replicates, by just deleting the columns of the variables *A*, *C* and *E* of the factorial design in table 2, without the need to carry out new experiments. Reintroducing these eight values for a 2^2 factorial design, the calculated standard deviation of each effect was 12.3 and for each coefficient 6.2, respectively (table 2).

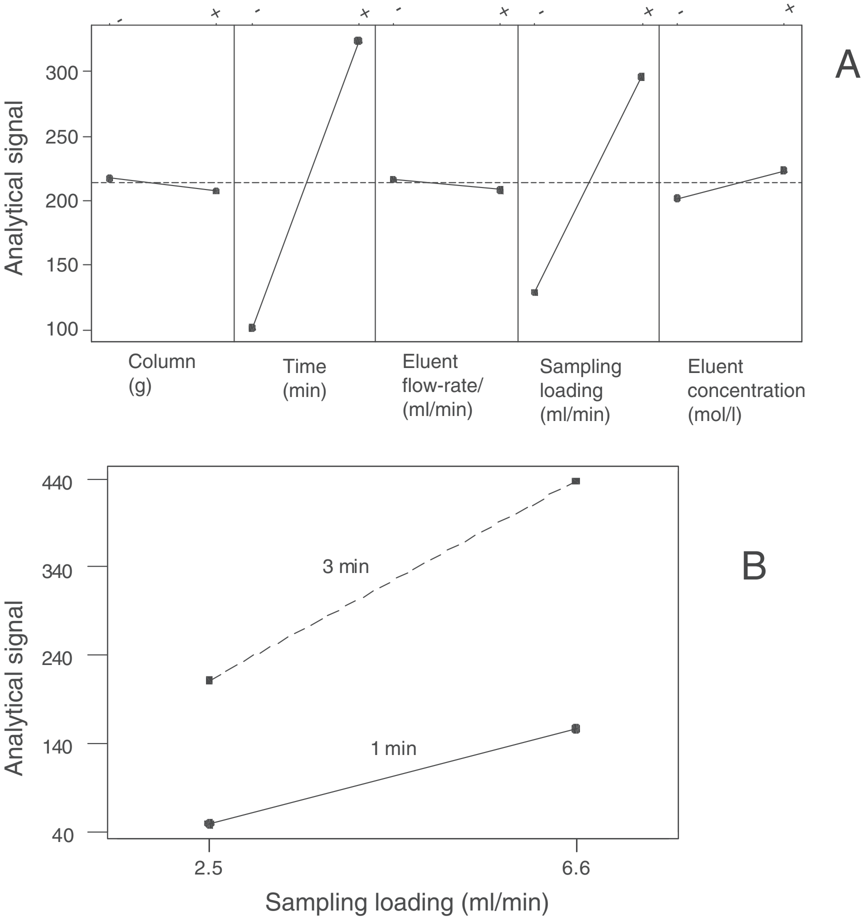


Figure 4. (A) Variation of analytical signal with all variables, mass of adsorbent present in the column, time of preconcentration, eluent flow rate, sampling loading, eluent concentration. (B) Analytical signal in the function of sampling loading at two different times of preconcentration. The analytical signal values are multiplied by 1000.

Figure 4A shows the variation in analytical signal in function of all tested factors. The slope of the lines when the levels of the variables changed from this lower level (−) to its higher level (+) indicates the significance of each individual variable on the overall process. As can be seen, the slope of the mass of adsorbent present in the column (*A*) and eluent concentration (*C*) are slightly negative, and the slope of eluent concentration is slightly positive, indicating that these variables are not significant in the overall optimization of copper preconcentration. On the other hand, the slopes of the variables' time of preconcentration (*B*) as well as analyte loading (*D*) are significantly positive. This graph shows only the individual variables on the overall optimization of the system. Figure 4B shows the change in analytical signal with sampling loading for two different times of preconcentration (1 and 3 min). As can be seen at 3 min, the slope of the line for the variation of the analytical signal with the sampling loading is higher than at 1 min, indicating that there is an interaction between the sampling loading (*D*) and the time of preconcentration (*B*). It should be stressed that this

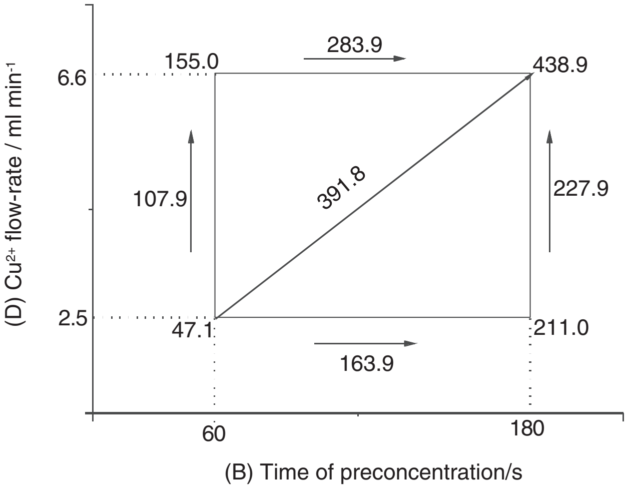


Figure 5. Graphical interpretation of simplified 2^2 factorial design for Cu^{2+} preconcentration. The analytical signal values have been multiplied by 1000.

interaction between the variables *B* and *D* could only be evaluated using the factorial design. In a univariate study of these variables, this interaction would never be noted [33, 40, 41].

The amount of sorbent in the minicolumn (*A*) was not significant because the AMPSG shows a high adsorption capacity $0.84\,\mathrm{mmol\,g^{-1}}$ [42], and the total amount of Cu^{2+} that percolated the minicolumn did not surpass the total capacity of the minicolumn. This conclusion was also confirmed by the time of preconcentration (*B*), when the time of preconcentration was varied from 60 (level −) to 180 s (level +), the analytical signal augmented by 4.5 times (47.1×10^{-3} to 211.0×10^{-3}) when the analyte flow rate (*D*) was kept at its minimum value and 2.8 times (155.0×10^{-3} to 438.9×10^{-3}) when the analyte flow rate was kept at its maximum value (see figure 5). If the adsorption capacity of the column were surpassed, the increase in the preconcentration time would not promote an increase in the analytical signal. In addition, to confirm the high adsorption capacity of the adsorbent to Cu^{2+}, the analyte flow rate also reinforces to this statement. When the Cu^{2+} flow rate was increased from 2.5 (level −) to $6.5\,\mathrm{mL\,min^{-1}}$ (level +), the analytical signal increased by a factor of 3.3 (47.1×10^{-3} to 155.0×10^{-3}) when the preconcentration time was kept at this minimum value, and the analytical signal increased by 2.1 factor (211.0×10^{-3} to 438.9×10^{-3}) when the preconcentration time was kept at this maximum value (figure 5). The more analyte percolates the column, the higher the analytical signal obtained, until the sorbent is saturated with the analyte. As the adsorption capacity of the minicolumn was not surpassed, the analytical signal increased by increasing both the Cu^{2+} flow rate as well as the loading time. By changing both the preconcentration time and the analyte flow rate from these respective low levels (− −) to their high levels (+ +), the analytical signal increased 9.3-fold (47.1×10^{-3} to 438.9×10^{-3}) (see figure 5). This interpretation shows the effect of interaction of the preconcentration time factor (*B*) with the Cu^{2+} flow-rate factor (*D*).

The eluent composition (*E*) and its flow rate (*C*) did not have any significant effects on the overall optimization of the preconcentration system, and their values were kept at the minimum level ($0.2\,\text{mol}\,\text{L}^{-1}$ of HNO_3 and $1.6\,\text{mL}\,\text{min}^{-1}$, respectively) so that they could be used in the flow-injection preconcentration system.

Using all the optimized conditions of the preconcentration of Cu^{2+} on the AMPSG sorbent, the analytical curve obtained for Cu^{2+} solution ranging from 50 to $300\,\text{ng}\,\text{mL}^{-1}$ was linear according to the equation $A = 9.12 \times 10^{-2} + 2.97 \times 10^{-3}$ $[Cu^{2+}]$, $r = 0.99913$, where Cu^{2+} concentrations are expressed in $\text{ng}\,\text{mL}^{-1}$.

The preconcentration factor obtained was 77, and it was calculated by the relation of the slope of preconcentration curve divided by the slope of the curve without preconcentration.

For determining the detection limit of the method, 20 measurements of the blank solution (0.2% HNO_3, pH 1) were performed (average absorbance of the blank, 0.053, RSD% 5.55%). The detection limit of the method defined as $3S$/slope analytical curve, where S is the standard deviation of determination $n = 20$, was $3.0\,\text{ng}\,\text{mL}^{-1}$. The quantification limit ($10S$/slope analytical curve) was $9.9\,\text{ng}\,\text{mL}^{-1}$ ($n = 20$). The detection limit of the method could be improved if ultra-pure buffer reagents were used, although this did not preclude the copper determination in the water samples.

The sample throughput obtained was 15 determinations per hour (180 s preconcentration time, 30 s elution time, 30 s regeneration of the column).

3.3 *Interference studies*

The effect of several elements on the sorption of $250\,\text{ng}\,\text{mL}^{-1}$ Cu^{2+} on the AMPSG sorbent (0.1002 g) was investigated. The recovery value is defined as 100 times the relationship between the signal of Cu^{2+} plus concomitants divided by the signal of Cu^{2+} alone. The tolerance level was established at 100 ± 5% as a reference. The Na^+ and K^+ ions could be tolerated up to $5000\,\text{mg}\,\text{L}^{-1}$, Mg^{2+} up to $500\,\text{mg}\,\text{L}^{-1}$, Ca^{2+} up to $1000\,\text{mg}\,\text{L}^{-1}$, Fe^{3+} up $50\,\text{mg}\,\text{L}^{-1}$, to Mn^{2+} up to $20\,\text{mg}\,\text{L}^{-1}$, Ni^{2+} up to $10\,\text{mg}\,\text{L}^{-1}$, Zn^{2+} up to $10\,\text{mg}\,\text{L}^{-1}$, Al^{3+} up to $20\,\text{mg}\,\text{L}^{-1}$, Ba^{2+} up to $20\,\text{mg}\,\text{L}^{-1}$, Co^{2+} up to $20\,\text{mg}\,\text{L}^{-1}$, and Cr^{3+} up to $20\,\text{mg}\,\text{L}^{-1}$. Based on these results, it can be concluded that the method could be successfully applied to the determination of Cu^{2+} in natural waters and brackish waters, since the concentrations of the concomitant species in these kinds of sample are usually lower than the proposed preconcentration method can tolerate [47].

3.4 *Determination of Cu^{2+} in water samples using the proposed preconcentration system*

The proposed preconcentration system using AMPSG as a sorbent was used for the determination of Cu^{2+} ($n = 5$) in four synthetic brackish waters (table 3). The percentages of Cu^{2+} recovery ranged from 95.8 to 103.3%, and these values are supported by the individual interference studies presented in the previous section.

In addition, the system was also used for copper determination in 14 ordinary natural water samples, and one water reference material was used (table 4), in order to achieve the accuracy of the preconcentration procedure. In addition, a recovery study was also carried out by spiking the water samples with $50\,\text{ng}\,\text{mL}^{-1}$ Cu^{2+}. The recoveries ranged from 95.4 to 104.7%.

Based on the results of tables 3 and 4, it can be inferred that Cu^{2+} can be successfully determined in natural and brackish waters with a good accuracy, employing a simple preconcentration system using AMPSG as a sorbent and spectrophotometric detection.

Table 3. Deternination of loaded and found values: Cu^{2+} recovery percentage in brackish water samples ($n = 5$) and several cationic species detected (M^{n+}) during the sorption process.

	$[Cu^{2+}]$/ng mL^{-1} water sample			
	I	II	III	IV
Cu^{2+}				
Loaded	60.0	100.0	200.0	250.0
Found	62.0	98.2	195.0	239.6
Recovery (%)	103.3	98.2	97.5	95.8
M^{n+}/mg L^{-1}				
Na^+	20.0	14.0	40.0	1000
K^+	11.0	22.0	16.0	500
Ca^{2+}	7.0	7.60	17.0	1000
Mg^{2+}	6.0	30.0	8.40	300
Al^{3+}	1.50	0.70	0.40	1.20
Ba^{2+}	3.00	2.50	1.50	2.00
Co^{2+}	0.40	0.50	0.67	0.08
Cr^{3+}	0.24	0.24	0.50	0.50
Fe^{2+}	1.70	1.80	2.50	2.00
Mn^{2+}	0.22	1.00	1.20	1.00
Ni^{2+}	0.09	0.24	0.60	0.35
Zn^{2+}	0.12	0.30	0.80	0.40

Table 4. Determination of Cu^{2+} in water samples ($n = 5$) using an on-line preconcentration system.[a]

Samples	$[Cu^{2+}] \pm S$/ng mL^{-1}	Rec/%
NIST 1640[b]	85.6 ± 1.7	100.1
Tap water	119.3 ± 2.0	96.1
Spring water	219.6 ± 2.7	101.1
Stream water	203.6 ± 1.7	96.3
Streamlet water	144.8 ± 2.8	102.6
Countryside river water	83.3 ± 2.2	101.4
Urban river water	225.8 ± 2.7	96.6
Well water I	232.4 ± 3.0	99.2
Well water II	211.3 ± 2.2	104.7
Waste water I[c]	2232 ± 15	97.5
Waste water II[c]	2782 ± 22	95.4
Waste water III[c]	1046 ± 15	100.3
Waste water IV[c]	2105 ± 27	99.6
Waste water V[c]	1544 ± 30	98.1
Waste water VI[c]	1354 ± 27	100.4

[a] The results are expressed as average value ± standard deviation S ($n = 5$) and the percentage recovery (Rec). The samples were spiked with 50 ng mL^{-1} Cu^{2+}; [b] certified value: 85.2 ± 0.9 ng mL^{-1}; [c] dilution factor: 10.0.

4. Conclusion

A simple flow preconcentration system for the determination of Cu^{2+} using DDTC as chromogenic agent and AMPSG as a sorbent was successfully used for the determination of the analyte in natural and brackish water samples. A full factorial design 2^5 for optimization of the analytical conditions for Cu^{2+} preconcentration showed that only the preconcentration time and analyte loading and its respective interaction

Table 5. Comparison among several flow-injection preconcentration procedures for copper(II) determination.[a]

Sorbent	Detector	DL (ng mL^{-1})	PT	EF	Method features	Reference
Poly(aminophosphonic acid) chelating resin	FAAS	1.6	75 s	19	No	20
3-(1-Imidazoyl)propyl grafted silica gel	FAAS	0.4	90 s	25.8	Interference from $20\,\text{mg L}^{-1}$ Fe^{3+}, $2\,\text{mg L}^{-1}$ Zn^{2+} and $10\,\text{mg L}^{-1}$ Mg^{2+}	25
Amberlite XAD-4 impregnated with 1-(2-pyridyl-azo)-2-naphtol	FAAS	0.06	50 min	30	Lifetime of the column 10 cycles	27
Unloaded polyurethane foam	FAAS	0.2	60 s	170	Sorption capacity 4.0 mg of Cu^{2+} per gram of sorbent	28
C18- silica gel	FAAS	2.0	60 s	19	Interference from $1.0\,\text{mg L}^{-1}$ Fe^{3+}	29
Amberlite XAD-2 impregnated with calmagite	FAAS	0.15	3 min	32	Interference from Fe^{3+}, Cr^{6+}	30
Amberlite XAD-2 functionalized with 3,4-dihidroxibenzoic acid	FAAS	0.27	2 min	33	Interference from $0.90\,\text{mg L}^{-1}$ of Zn^{2+} and Fe^{3+}	31
Brown alga impregnating silica gel	ICP-AES	0.6	2 min	13	Lifetime of the column 30 cycles	35
TiO_2 impregnated with 1-(2-pyridyl-azo)-2-naphtol	ICP-AES	2.8	75 min	75	Suffers severe interference of $10.0\,\text{mg L}^{-1}$ of Na^+, K^+, Ca^{2+}, and Mg^{2+}	36
2-Aminomethylpyrydine grafted silica-gel	VIS $\lambda = 460\,\text{nm}$	3.0	3 min	77	Sorption capacity $53.4\,\text{mg g}^{-1}$ of sorbent	This work

[a] PT: preconcentration time; EF: enrichment factor; DL: detection limit.

were significant. Using the best conditions, the copper preconcentration factor achieved with the AMPSG was 77, using a preconcentration period of just 3 min; in addition, the detection limit of 3.0 ng mL^{-1} was also attained. Therefore, it is possible to determine copper in environmental water samples with a detection limit better than that achieved with graphite furnace atomic spectrometry (6.4 ng mL^{-1}) [48], although the cost of implementing the proposed procedure is less than US$4000. A graphite furnace spectrometer costs no less than US$50,000. Therefore, when the use of flow preconcentration systems using good sorbents as AMPSG becomes widespread, the analysis of a low concentration of elements could be carried out in any simple analytical laboratory.

Table 5 lists several flow-injection preconcentration procedures involving different kinds of adsorbents, using FAAS and ICP-AES detectors. As can be seen, the previously published preconcentration procedures achieved detection limits better than this present work, whose determination was based on colorimetric determination of Cu^{2+} with DDTC. The main reason for this achievement is the better detection power and selectivity of FAAS and ICP-AES in relation to colorimetric detectors. On the other hand, the best characteristics of the adsorbent are those obtained with the organofuctionalized silica gel [25] and the proposed one. Adsorbents whose adsorption group is not chemically bonded to the support have a shorter lifetime [27, 30, 35, 36], where the active groups responsible for the sorption are usually leached out after a few preconcentration cycles; also, these procedures are prone to suffer interference from concomitant species present in the samples, due to the low adsorption capacities. Preconcentration procedures involving the use of organic solvents [28, 29] are not suitable nowadays, because many of these procedures use toxic solvents [28] that are harmful to humans, besides increasing the cost of the procedure [29]. Adsorbents with a low adsorption capacity [25, 31] also suffer from interference caused by the main concomitants of real samples, thus limiting their use in more complex samples [25, 31]. It is important to point out that the determination of Cu^{2+} in natural water samples employing the flow-injection preconcentration system with colorimetric detection was only possible because the highest adsorption capacity of 2-aminomethylpyridine grafted silica gel (0.84 mmol g^{-1}) was not surpassed during the preconcentration procedure.

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