

Use of 1,3-diaminepropane-3-propyl grafted onto a silica gel as a sorbent for flow-injection spectrophotometric determination of copper (II) in digests of biological materials and natural waters

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Abstract

The 1,3-diaminepropane-3-propyl-anchored silica gel (DAPPS) was successfully employed as a sorbent in a spectrophotometric flow system for the preconcentration of Cu²⁺ in digests of biological materials (maize powder, soybean, citrus leaves, corn stalks) as well as water samples (river, stream, streamlet, springwater and well). The system presented a minicolumn packed with DAPPS, where the sample solution was passed through it for a period of time, and subsequently, an eluent solution, stripped-out the retained analyte which was further determined with DDTc at 460 nm.

The better preconcentration conditions utilized were: 120 s loading, 60 s elution, 30 s regeneration of the column, loading flow rate 6.5 ml min⁻¹, buffer solution for the preconcentration and regeneration of the column-borate buffer pH 8.5, elution flow rate 2.3 ml min⁻¹, time of elution 60 s, eluent composition, 0.4 mol l⁻¹ HNO₃. Under these conditions, the preconcentration factor obtained was 36, and the detection limit achieved was 8.4 ng ml⁻¹ in water samples and 0.84 µg g⁻¹ in biological material. The maximum adsorption capacity of DAPPS to Cu²⁺ was 0.49 mmol g⁻¹ (31.1 mg g⁻¹) obtained in a batch system.

The recovery of copper in the water samples ranged from 96.9 to 102.4% and in the biological materials ranged from 97.0 to 102.6%.

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1. Introduction

Spectrophotometry is not usually employed for the determination of analytes in real samples because it suffers severe interferences of concomitants present in the sample matrix. Therefore, its use for determination of analytes in real samples requires a separation procedure. Among the several separation procedures, solid-phase extraction is the most attractive because it does not require the use of hazardous solvents, which generates large volumes of waste that need to be treated subsequently [1], besides being a time-consuming procedure that leads to a decrease of the sampling throughput. Modified silica with different organic groups is one

of the most successful sorbents employed in the analytical laboratories, because the silica supports does not swells or shrinks such as the polymeric resin [2], and unmodified natural occurrence materials [3]; allows the modified silica sorbent to be used during several cycles of preconcentration, because the retention process (adsorption, chelation, ion exchange) are reversible [4,5]; the modified silica may be employed in aqueous and organic solvents media [4,5]; they present good thermal stability [4,5] and appropriated accessibility of the ions to the attached chelating groups; in addition, the modified silica gel exhibits sorption capacities higher than polymeric resins, because the number of organic molecules immobilized on the support surface is higher, allowing high preconcentration factors [1,4,5].

The organofunctionalized silica may be obtained by just impregnating the support with an organic substance [6,7],

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by sol–gel method [8–11], or by grafting an organic pendant group to silica [12–19]. The first procedure does not produce a suitable material for the preconcentration of metals because surface coverage of organic substances in the silica support is low, leading as consequence to low preconcentration factors, in addition, the achieved analytical signal decreases with the number of preconcentration cycles. The silica obtained from the sol–gel method for preconcentration of metals has few analytical applications to real samples [9,11], because this kind of silica synthesis leads to a microporous materials which are not compatible for reversible preconcentration systems [10]. The grafting of organic groups to silica material is the most efficient method for modeling sorbents with desired chelating properties [4,5,20].

Flow-injection preconcentration systems using mini-columns packed with a suitable sorbent for the preconcentration of several analytes in the most different kinds of sample has been successfully employed [18,19,21–26]. This procedure presents several advantages over conventional column preconcentration procedures such as diminution of total time of preconcentration of several hours to carry out just one cycle [21–26] to just few minutes which increases the sampling throughput remarkably; higher repeatability of the measurements acquired with flow systems [27]; diminution of risk of contamination because the manual operation is diminished [21,22,27]. However, flow-injection preconcentration systems present several advantages; it has been coupled to mainly to flame atomic absorption spectrometry (FAAS) [18,19,24,28–32] and inductively coupled plasma atomic emission spectrometry (ICP-AES) [23–26,33]. On the other hand, the applications of flow-injection preconcentration system coupled to spectrophotometry for the determination of metallic ions are scarce [34], there being a need to be explored more extensively. In order to achieve this application, more selective sorbents should be employed, since spectrophotometry alone, does not provide enough selectivity for the determination of analytes in real sample matrices.

In this paper, 1,3-diaminepropane-3-propylsilica (DAPPS) as a selective copper (II) sorbent was successfully employed for its preconcentration in digests of botanic material as well as natural water samples. It was observed that at pH 8, measured at the effluent of the column, the ions Fe^{3+} , Mn^{2+} , Zn^{2+} , Al^{3+} , Cr^{3+} , Co^{2+} , Ni^{2+} were not sorbed on DAPPS.

2. Experimental

2.1. Instruments

A 600 S Femto spectrophotometer (São Paulo, SP, Brazil) provided with 150 μl flow cell (Femto) and a serial port RS232C connected to AMD K6II 350 MHz personal computer for data acquisition were employed 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 the solutions in the flow system. For the pH measurements, a pH/mV hand-held meter handylab 1 Schott (Mainz, Germany) provided with combined glass electrode model BlueLine 23 pH was used.

2.2. Reagents and solutions

Doubly distilled water was employed throughout. Solutions containing 0.1–1.0 mol l^{-1} of nitric acid (Merck, Rio de Janeiro, RJ, Brazil) was employed as eluent. A 1.00 g l^{-1} copper (II) stock solution was prepared dissolving 0.5 g of metallic copper, weight with the precision of the tenth of milligram (Merck, Rio de Janeiro, RJ, Brazil) in 10 ml of (1 + 1, v/v) nitric acid–water and this solution was quantitatively transferred to a 500-ml calibrated flask and the volume was completed to the mark with distilled water. The calibration solutions within 25–400 ng ml^{-1} (preconcentration) and 1000–5000 ng ml^{-1} (without preconcentration) of Cu^{2+} range were prepared by suitable serial dilution of the stock solution with doubly distilled water and adjusting the final acidity to pH 1.0 with HNO_3 .

A 0.15% (m/v) of sodium diethyldithiocarbamate (DDTC; Merck, Darmstadt, Germany) solution was daily prepared 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), and then filtered to 250 ml volumetric flask and the volume completed to the mark with distilled water.

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

For the interference studies, the following salts of elements were employed: NaCl (Merck, Rio de Janeiro, RJ, Brazil), $\text{NaH}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$ (Reagen, Rio de Janeiro, RJ, Brazil), $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ (Merck, Rio de Janeiro, RJ, Brazil), KCl (Merck, Rio de Janeiro, RJ, Brazil), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (Merck, Rio de Janeiro, RJ, Brazil), $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (Merck, Rio de Janeiro, RJ, Brazil), $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (Merck, Rio de Janeiro, RJ, Brazil), $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ (Vetec, Rio de Janeiro, RJ, Brazil), $\text{Ba}(\text{NO}_3)_2$ (Reagen, Rio de Janeiro, RJ, Brazil), $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ (Vetec, Rio de Janeiro, RJ, Brazil), $\text{FeSO}_4 \cdot \text{NH}_4\text{SO}_4 \cdot 6\text{H}_2\text{O}$ (Merck, Rio de Janeiro, RJ, Brazil), $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (Reagen, Rio de Janeiro, RJ, Brazil), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (Nuclear, Diadema, SP, Brazil), $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ (Reagen, Rio de Janeiro, RJ, Brazil).

2.3. Synthesis and characterization of the 1,3-diaminepropane-3-propylsilica (DAPPS)

An amount of 15 mmol of 1,3-diaminepropane was first activated using 15 mmol of sodium hydride in 10 ml

of a mixture of aprotic solvent (toluene:tetrahydrofuran) (1:1) (Merck, Hohenbrunn, Germany) for 30 min, and then 15 mmol of 3-chloropropyltrimethoxysilane (CPTMS, Acros Organics, New Jersey, USA) was added. The mixture was stirred under argon at solvent-reflux temperature for a period of 5 h. The solution was then centrifuged, to eliminate the byproduct sodium chloride. The product of reaction, 1,3-diaminepropane-3-propyltrimethoxysilane (AMSIL) was then used as organic precursor reagent for the silica grafting reaction.

Silica gel (Merck, Darmstadt, Germany) 0.02–0.05 mm of particle size and a surface area of $250 \text{ m}^2 \text{ g}^{-1}$ was activated at 150°C under vacuum (10^{-1} Pa) for 5 h. The AMSIL was dissolved in 300 ml of toluene and the activated silica (10 g) was then added. The mixture was stirred for 48 h under argon at solvent-reflux temperature. The modified silica was filtered under argon in a Schlenk apparatus, washed with toluene, hexane, ethyl alcohol, doubly distilled water and ethyl ether (supplied by Merck, Hohenbrunn, Germany). The resulting sorbent 1,3-diaminepropane-3-propylsilica (DAPPS) was dried for 2 h under vacuum at 120°C . The DAPPS presented a specific surface area $230 \text{ m}^2 \text{ g}^{-1}$ and a coverage of silica surface of 0.70 mmol of 1,3-diaminepropane groups per gram of sorbent.

2.4. Samples

Ordinary samples of maize powder, soybean, citrus leaves, corn stalks and water samples (river, stream, streamlet, springwater and well) were employed. The following reference materials were employed for attaining the accuracy of the method, Chlorella (NIES-CRM-03) from National Institute for Environmental Studies (NIES, Ibaraki, Japan), Apple Leaves (NIST-SRM 1515) and Trace Elements in Natural Water (NIST-1640) from National Institute of Standards and Technology (NIST, Gaithersburg, USA).

The ordinary water samples were filtered with Whatman paper pH adjusted to 1.0 with HNO_3 and analyzed employing the procedure of preconcentration.

The solid samples were digested in triplicate with nitric acid plus hydrogen peroxide using a block digester (Tecnal, Piracicaba, SP, Brazil) according to the following procedure [37]: 0.5 g of sample was accurately weighed with a precision of the tenth of milligram, and transferred to a 25-cm long borosilicate digestion tube (25 mm internal diameter), followed by the addition of 5.0 ml concentrated HNO_3 ; the mixture was digested at temperatures not exceeding 160°C for 8 h. Afterwards, the digestion tubes were stand to reach the room temperature. Thereafter, 2.0 ml of 30% (m/m) H_2O_2 (Merck, Rio de Janeiro, Brazil) were dropwise added, and the block digester was heated to 100°C for about 30 min, obtaining a colorless solution. The excess of HNO_3 was driven off by gently heating, and during this procedure water was added to the tubes to avoid dryness. The volume was

made up to 50 ml with water (1.0%, v/v, HNO_3 final acidity) and the resulting solution was analyzed in the flow system.

2.5. Isotherms of adsorption of Cu^{2+} on DAPPS

A 10-ml of sample solution containing 7.87×10^{-6} to $1.55 \times 10^{-2} \text{ mol l}^{-1}$ of Cu^{2+} solution plus 10.0 ml of borate buffer solution (pH 8.0) was transferred to a 50-ml conical polyethylene flask containing 20 mg of sorbent. These flasks were placed in a horizontal shaker and agitated for 180 min in order to adsorb the analyte. Subsequently, the solid phase was separated from aqueous phase by filtration, being the aqueous phase retained for analysis.

The Cu^{2+} contents, which were not retained in the sorbent, were spectrophotometrically determined, using 0.15% (m/v) sodium diethyldithiocarbamate (DDTC) as chromogenic agent. The measurements were carried out using a FEMTO 600 S spectrophotometer, according to the following procedure: an aliquot of 1.00 ml of aqueous phase and/or analyte standard solution plus 1.00 ml of chromogenic reagent plus 1.00 ml of water were added to a Hellma glass cuvette (10 mm of optical path). The analyte solutions with concentration higher than those cited above were properly diluted with distilled water. The metal ion adsorption capacity of the solid phase (N_f), obtained in the saturation plateau, was calculated by applying the equation $N_f = (C_s - C_e) \times V/m$, where C_s is the initial metal concentration, C_e the metal concentration at the equilibrium found in the solution in equilibrium with the solid phase, V the volume of the solution put with the sorbent, and m the mass of the solid phase.

2.6. Flow system

For the determination of Cu^{2+} in the samples, a homemade injection valve made of Perspex was used [38]. The glass minicolumn (4 cm, ID 2.5 mm, 0.11 g of sorbent) was placed in the central position of the injection valve and was utilized 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 Fig. 1. Initially, for developing the method, the system employed a loop instead of a minicolumn. In the preconcentration position (position I), the sample solution ($25\text{--}400 \text{ ng ml}^{-1} \text{ Cu}^{2+}$, 6.5 ml min^{-1} , pH 1) and the buffer solution B_1 (0.8 mol l^{-1} borate buffer, pH 8.5; 4.3 ml min^{-1}) are merged in the confluence point X, subsequently these solutions are mixed in the coil C_1 ($100 \mu\text{l}$) and directed to the minicolumn, for sorbing the analyte present in the sample solution and the aqueous phase is directed to the waste W_s (pH 8 measured at the effluent of column). In the second channel, the carrier solution E ($0.4 \text{ mol l}^{-1} \text{ HNO}_3$; 2.3 ml min^{-1}) is being pumped to the system where at confluence point Y it merges with B_2 buffer solution (0.8 mol l^{-1} borate buffer, pH 8.5; 2.3 ml min^{-1}) and mixed in the coil mixer C_2 ($200 \mu\text{l}$), then the buffered sample zone reaches the

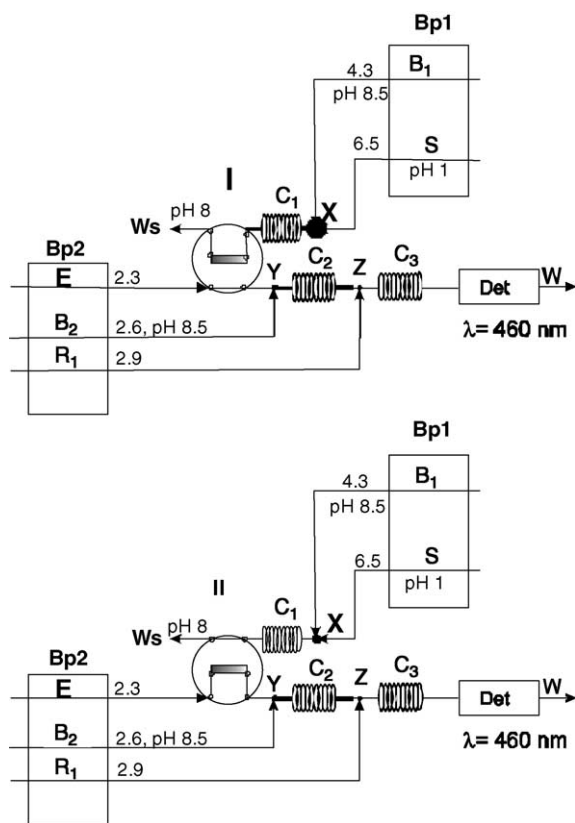


Fig. 1. Manifold employed for the determination of Cu^{2+} in the samples. I, preconcentration position; II, eluting position. B_1 , 0.8 mol l^{-1} borate buffer solution; S, sample solution (pH 1); W and W_s , wastes; E, eluent 0.4 mol l^{-1} HNO_3 ; R_1 , 0.15% DDTC; B_2 , 0.8 mol l^{-1} borate buffer solution; C_1 , C_2 and C_3 , coil reactors, 100, 100, and $200 \mu\text{l}$, respectively; Bp1 and Bp2, peristaltic pumps; Det, spectrophotometric flow cell, $150 \mu\text{l}$, $\lambda = 460 \text{ nm}$. X, Y, Z, confluence points. The flow rates are expressed in ml min^{-1} .

confluence point Z, where it merges with reagent R_1 (0.15% , m/v , DDTC; 2.9 ml min^{-1}) and subsequently it reaches the mixture coil C_3 ($200 \mu\text{l}$) and this sampling zone passes in the flow cell ($150 \mu\text{l}$) of the spectrophotometer (460 nm) producing the baseline and subsequently this solution is directed to the waste (W). After 120 s of preconcentration period, unless otherwise stated, the injection valve is positioned in the elution position (position II), and the minicolumn is inserted in the eluent line, eluting the retained analyte at flow rate of 2.3 ml min^{-1} . At the confluence point Y, the sample zone merges with B_2 buffer solution and mixed in the coil mixer C_2 , then the buffered sample zone reaches the confluence point Z where it merges with the chromogenic reagent and then mixed in the coil mixer C_3 ($200 \mu\text{l}$) forming the $\text{Cu}(\text{DDTC})_2$ complex [39]. This zone is subsequently directed to the flow cell of the spectrophotometer (460 nm), producing a transient signal, and finally this zone is directed to the waste (W). During the elution and reaction color developing step, the sample solution is replaced by water and this plus the B_1 buffer solution are directed to the waste (W_s). After the suitable elution time (60 s, unless otherwise stated),

the injection valve is positioned in the position I, and the column is reconditioned with water and buffer solution. After a period (30 s, unless otherwise stated), the water channel is substituted by sample solution and a further preconcentration cycle is carried out. The total time for a preconcentration cycle and regeneration of the sorbent is 210 s, unless otherwise stated.

3. Results and discussion

3.1. Optimization of flow system conditions without preconcentration

The first variable investigated for the optimization of the reaction between Cu^{2+} and DDTC was the pH. Several $0.5\text{--}2.0 \text{ mol l}^{-1}$ buffer solutions, in order to support higher acidic concentration of real samples, with pH ranging from 4.5 to 10.5 were tested. It was observed that the pH interval for Cu^{2+} determination with DDTC is large ranging from 5.5 to 9.0. Then at first approach, 0.8 mol l^{-1} borate buffer with pH adjusted to 8.5 was chosen for the color forming reaction. The flow rate of the buffer solution was also investigated from 2.0 to 5.0 ml min^{-1} . It was observed that the best sensitivity occurred at flow rates of 2.6 ml min^{-1} .

The concentration of DDTC was fixed at 0.15% (m/v) 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 2.6 to 4.0 ml/min did not presented significant changes in the analytical signals. A flow rate of 2.9 ml min^{-1} DDTC was employed throughout this work.

The dimensions of coil reaction C_2 and C_3 were also investigated, and the dimensions that promoted the highest and sharpest analytical signal was 100 and $200 \mu\text{l}$, respectively. The C_2 reaction coil serves to buffer the sample zone previously to the addition of the chromogenic agent. After adding the DDTC to the buffered sample zone, this reaction zone is 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 25 s to return the baseline after the appearing of the maximum of the peak.

The sample loop was varied from 75 to $500 \mu\text{l}$. As a compromise between the sensitivity and sharpness of the peak profile, a sample loop of $375 \mu\text{l}$ (75 cm) was chosen. For sample loop of $500 \mu\text{l}$ (100 cm) the analytical signal achieved was broaden. Also it was observed that the time for the analytical signal to return to the baseline after the maximum of peak appearance was more than 25 s.

The last variable investigated for the optimization of the flow system without preconcentration, for the color forming reaction was the concentration of the HNO_3 , used as carrier solution, ranging from 0.1 to 1.0 mol l^{-1} , and also its flow rate. The best conditions were obtained when 0.4 mol l^{-1} HNO_3 at flow rate of 2.6 ml min^{-1} were employed. Under these conditions the analytical calibration curve, without pre-

concentration, was performed obtaining the following equation $A = -4.24 \times 10^{-3} + 6.06 \times 10^{-5} [\text{Cu}^{2+}]$, $r = 0.99801$ for copper (II) concentration expressed in ng ml^{-1} .

3.2. Optimization of the flow system for the preconcentration and figures of merit

The amount of Cu^{2+} that percolates the column is an important variable to be optimized. The more analyte percolates the column, higher is the analytical signal obtained until the saturation of the sorbent is attained [21,22]. In Fig. 2A is shown the effect of sample flow rate on the analytical signal. It was observed that 6.5 ml min^{-1} of 250 ng ml^{-1} Cu^{2+} passing through the column, the highest analytical signal was achieved, being this sample flow rate chosen for improving this work.

The DAPPS sorbent presents two amino groups which chelate the metal ion, as depicted in Scheme 1. The formation of five and six member rings chelates are thermodynamically favorable leading to a high complex formation constants (K_f), however, the conditional complex formation constant depends strongly on the pH the reaction [35,36]. In Fig. 2B is presented the effect of the pH, of the buffer solution in the exit of the minicolumn, on the sorption of Cu^{2+} on the DAPPS. As can be seen, the maximum signal was obtained, when the pH of the buffer solution in the exit of the minicolumn was fixed 7.0–8.5. Based on these results, the buffer solution employed for the preconcentration of Cu^{2+} in acidic water samples (pH 1) was 0.8 mol l^{-1} borate buffer pH adjusted 8.5, which allow a solution at the exit of the minicolumn with pH 8.

In Fig. 2C is presented the effect of the flow rate of the buffer solution (pH 8.5) on the sorption of Cu^{2+} on the DAPPS sorbent. It is observed that the higher sensitivity obtained was when the flow rate of the buffer solution was set at $3.4\text{--}4.5 \text{ ml min}^{-1}$. For lower flow rates, the buffer solution (pH 8.5) was not efficient for mixing with the sample solution (250 ng ml^{-1} Cu^{2+} , pH 1) flowing at 6.5 ml min^{-1} , in order to keep the amino groups of the sorbent non-protonated. It was also observed that, for flow rates lower than 2.7 ml min^{-1} of the buffer solution, the time for the regeneration of the sorbent for a subsequent preconcentration cycle should be increased, reducing the sample throughput. For flow rates higher than 4.8 ml min^{-1} , the dispersion was increased resulting in a decrease of the analytical signal [27]. As a compromise between sensitivity and sample throughput, the flow rate of the buffer solution for the preconcentration of Cu^{2+} was fixed at 4.3 ml min^{-1} .

Other important variable for the preconcentration of elements in flow system is the composition of the eluent [21,22]. In previous studies, using the flow system with sample loop, the best sample carrier used was 0.4 mol l^{-1} HNO_3 , which was suitable for removing the analyte from DAPPS column completely. Then the eluent was fixed as 0.4 mol l^{-1} HNO_3 .

The eluent flow rate was also optimized (Fig. 2D). As can be seen, the best eluent flow rate is $2\text{--}2.5 \text{ ml min}^{-1}$. For eluent flow rates lower than 1.8 ml min^{-1} the analytical signal

was significantly diminished, probably the amount of acid was not enough to strip-out all the retained analyte from the DAPPS minicolumn. For eluent flow rates higher than 2.9 ml min^{-1} the analytical signal also diminished, probably due to dilution of the analyte stripped out from the DAPPS sorbent [21,22]. By these reasons, the eluent flow rate was fixed at 2.3 ml min^{-1} .

The last variable to be studied was the time of preconcentration (Fig. 2E). The Cu^{2+} preconcentration was linearly increased with the time of preconcentration up to 180 s, after that the preconcentration was levelled off. In order to not decrease the sample throughput, the preconcentration time was fixed at 120 s. In this situation, the sample throughput was 17 measurements per hour.

Using all the optimized conditions of the preconcentration of Cu^{2+} on the DAPPS sorbent, the analytical curve for the analyte determination in water samples was achieved (Fig. 2F). The linearity was obtained for Cu^{2+} solution ranging from 50 to 400 ng ml^{-1} . For the copper determination in digests of biological materials, the linearity of the analytical curve ranged from 25 to 200 ng ml^{-1} .

The preconcentration factor obtained was 36 and the detection limit defined as $((\text{blank} + 3\sigma)/\text{slope analytical curve})$, where σ is the standard deviation of blank determination ($n = 20$) was 8.4 ng ml^{-1} . The quantification limit $((\text{blank} + 10\sigma)/\text{slope analytical curve})$ was 13.4 ng ml^{-1} ($n = 20$). Multiplying the detection limit and quantification limit by the dilution factor (100 ml g^{-1}), the detection and quantification limits attained for the biological materials were 0.84 and $1.34 \mu\text{g g}^{-1}$, respectively.

In order to evaluate the adsorption capacity of DAPPS sorbent for Cu^{2+} , solutions of this element were placed into contact under agitation with the DAPPS adsorbent for 180 min using a batch procedure. The maximum adsorption capacity of Cu^{2+} was 0.49 mmol g^{-1} (31.1 mg g^{-1}) (see Fig. 3).

3.3. Interferences and recoveries studies

As it is known that DDTTC form complexes with Cu^{2+} , Mn^{2+} , Zn^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Pb^{2+} , Cd^{2+} , Hg^{2+} [39], it is necessary to know if these ions will be retained in the DAPPS minicolumn. As the ions Pb^{2+} , Cd^{2+} and Hg^{2+} are always present in very lower concentrations than Cu^{2+} in biological materials and waters [40] these ions were not studied. In Fig. 4 is presented the sorption of 250 ng ml^{-1} Cu^{2+} , 5 mg l^{-1} Mn^{2+} , 5 mg l^{-1} Zn^{2+} , 50 mg l^{-1} Fe^{3+} , 5 mg ml^{-1} Co^{2+} , 5 mg ml^{-1} Ni^{2+} on DAPPS in function of the pH of the exit of the minicolumn, using 0.15% (m/v) DDTTC as chromogenic reagent, and monitoring the analytical signal at $\lambda = 460 \text{ nm}$, and all other Cu^{2+} optimized conditions. As can be seen at pH 8, all the tested concomitant species were not sorbed on DAPPS minicolumn. Therefore it is expected that the proposed method for Cu^{2+} preconcentration will be free from interferences with samples having up to 5 mg l^{-1} Mn^{2+} , 5 mg l^{-1} Zn^{2+} , 50 mg l^{-1} Fe^{3+} , 5 mg ml^{-1} Co^{2+} , 5 mg ml^{-1} Ni^{2+} .

A more extensive interference effects of other elements on the sorption of $250 \text{ ng ml}^{-1} \text{ Cu}^{2+}$ on the DAPPS sorbent (0.11 g) was also investigated (Fig. 5), employing the copper optimized conditions. 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 $100 \pm 5\%$ as a reference. The Na^+

and K^+ ions could be tolerated up to 5000 mg l^{-1} , Mg^{2+} up to 1000 mg l^{-1} , Ca^{2+} up to 500 mg l^{-1} , Al^{3+} up to 50 mg l^{-1} , Ba^{2+} up to 50 mg l^{-1} , Cr^{3+} up to 20 mg l^{-1} , SO_4^{2-} up to 500 g l^{-1} , PO_4^{3-} up to 200 g l^{-1} , and Cl^- up to 5000 g l^{-1} . Based on these results, it can be concluded that the method could be successfully applied to the determination of Cu^{2+} in digests of biological materials as well as natural waters, since

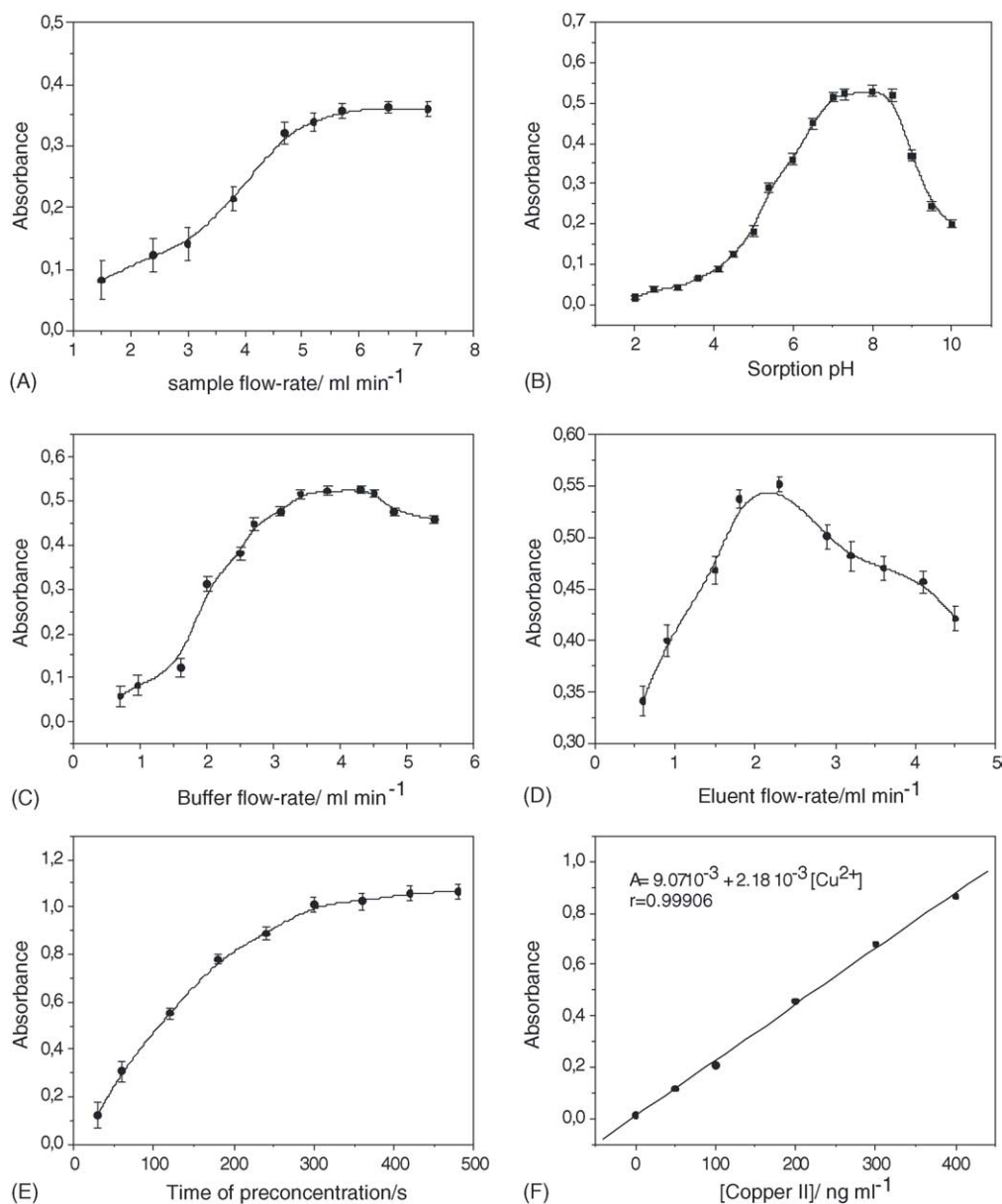
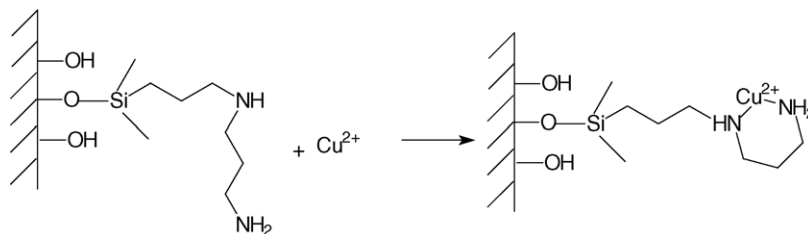


Fig. 2. Optimization of preconcentration flow system. Chromogenic agent $0.15\% \text{ DDTC}$, 2.9 ml min^{-1} ; buffer for color forming reaction, 0.8 mol l^{-1} borate buffer solution (pH 8.5), 2.6 ml min^{-1} ; (A) effect of sample flow rate, $250 \text{ ng ml}^{-1} \text{ Cu}^{2+}$; time of preconcentration 120 s; buffer solution pH 7.0; buffer flow rate, 3.4 ml min^{-1} ; eluent, $0.5 \text{ mol l}^{-1} \text{ HNO}_3$, 1.8 ml min^{-1} ; time of elution, 60 s; time of regeneration, 60 s. (B) Effect of pH on the sorption, $250 \text{ ng ml}^{-1} \text{ Cu}^{2+}$; sample flow rate, 6.5 ml min^{-1} ; time of preconcentration, 120 s; buffer flow rate, 3.4 ml min^{-1} ; eluent, $0.5 \text{ mol l}^{-1} \text{ HNO}_3$, 1.8 ml min^{-1} ; time of elution, 60 s; time of regeneration, 30 s. (C) Effect of the buffer flow rate for the copper preconcentration, $250 \text{ ng ml}^{-1} \text{ Cu}^{2+}$; sample flow rate, 6.5 ml min^{-1} ; time of preconcentration, 120 s; buffer solution pH 8.5; eluent, $0.5 \text{ mol l}^{-1} \text{ HNO}_3$, 1.8 ml min^{-1} ; time of elution, 60 s; time of regeneration 30 s. (D) Eluent flow rate, $250 \text{ ng ml}^{-1} \text{ Cu}^{2+}$; sample flow rate, 6.5 ml min^{-1} ; buffer solution pH 8.5; buffer flow rate, 4.3 ml min^{-1} ; time of preconcentration, 120 s; eluent, $0.4 \text{ mol l}^{-1} \text{ HNO}_3$; time of elution, 60 s; time of regeneration, 30 s; (E) Time of preconcentration, $250 \text{ ng ml}^{-1} \text{ Cu}^{2+}$; sample flow rate, 6.5 ml min^{-1} ; buffer solution pH 8.5; buffer flow rate, 4.3 ml min^{-1} ; eluent, $0.4 \text{ mol l}^{-1} \text{ HNO}_3$, 2.3 ml min^{-1} ; time of elution, 60 s; time of regeneration, 30 s. (F) Analytical curve, sample flow rate, 6.5 ml min^{-1} ; buffer solution pH 8.5; time of preconcentration, 120 s; buffer flow rate, 4.3 ml min^{-1} ; eluent, $0.4 \text{ mol l}^{-1} \text{ HNO}_3$, 2.3 ml min^{-1} ; time of elution, 60 s; time of regeneration, 30 s.

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

the concentration of the concomitant species in these kind of sample are usually lower than the proposed preconcentration method can tolerate [40].

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

The proposed preconcentration system using DAPPS as a sorbent was employed for the determination of Cu^{2+} ($n = 3$) in seven ordinary natural water samples and one water reference material (Table 1), which was employed in order to achieve the accuracy of the preconcentration procedure. In addition, a recovery study was also carried out, by spiking the natural water samples with $100 \text{ ng ml}^{-1} \text{ Cu}^{2+}$. The recoveries ranged from 96.9 to 102.4%.

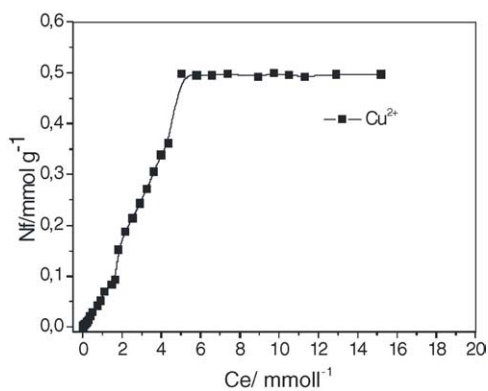
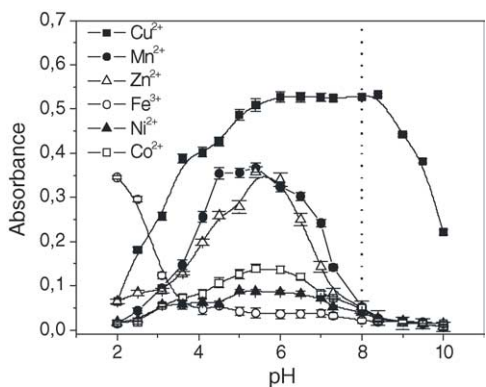
Fig. 3. Adsorption isotherm of Cu^{2+} on DAPPS. Time of agitation 180 min.

Fig. 4. Sorption of several metals on DAPPS in function of the pH.

Also, the preconcentration system using DAPPS as sorbent was also employed for the determination of Cu^{2+} in digests of biological materials (Table 2). The contents of copper were determined in four ordinary biological materials and for obtaining the accuracy of the method two biological

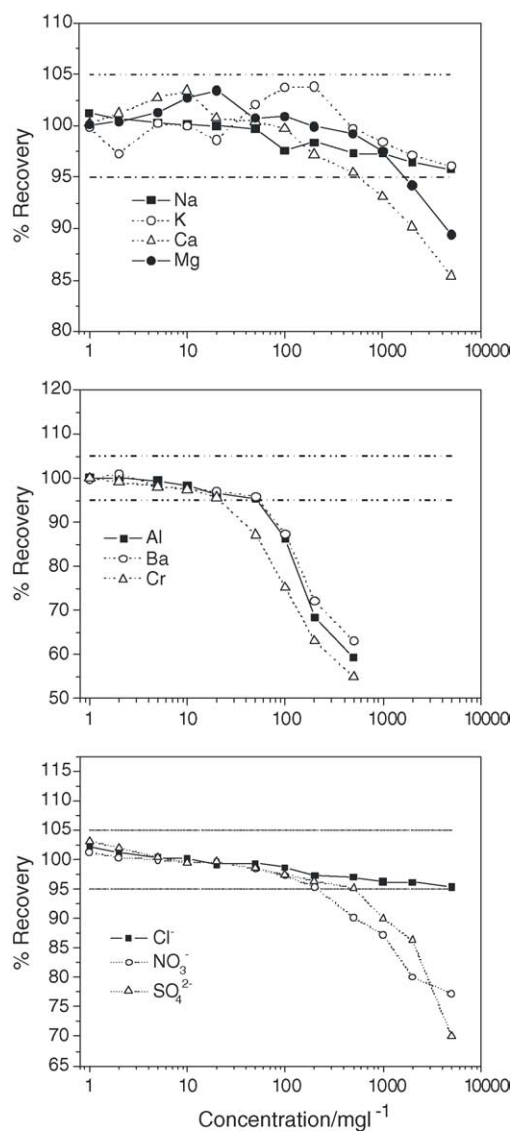
Fig. 5. Interference of several elements on the sorption of $250 \text{ ng ml}^{-1} \text{ Cu}^{2+}$ on the DAPPS sorbent. For details of the optimized conditions of flow system, see Fig. 2.

Table 1
Determination of Cu²⁺ in water samples (*n* = 3) employing an on-line preconcentration system

Samples	[Cu ²⁺] ± S.D. (ng ml ⁻¹)	Found with spike 100 ng ml ⁻¹	%Recovery
NIST 1640 ^a	85.6 ± 1.7	185.7 ± 2.7	100.1
Countryside river water	79.2 ± 2.9	179.1 ± 2.4	99.9
Urban river water	218.2 ± 3.4	311.4 ± 2.2	96.9
Streamlet water	160.0 ± 11.8	258.1 ± 2.4	98.9
Stream water	199.4 ± 1.6	304.2 ± 2.2	102.4
Springwater	216.0 ± 3.7	319.9 ± 1.4	101.8
Well water I	236.8 ± 3.7	342.0 ± 1.6	102.2
Well water II	206.1 ± 4.4	304.4 ± 1.7	99.2

Results are expressed as average value ± S.D. (*n* = 3). The samples were spiked with 100 ng ml⁻¹ Cu²⁺.

^a Certified value 85.2 ± 0.9 ng ml⁻¹.

Table 2
Determination of Cu²⁺ in biological materials (*n* = 3) employing an on-line preconcentration system

Samples	[Cu ²⁺] ± S.D. (μg g ⁻¹)	[Cu ²⁺] ± S.D. (ng ml ⁻¹)	Found with spike 50 ng ml ⁻¹	%Recovery
Maize powder	4.71 ± 0.16	47.13 ± 0.7	97.41 ± 0.20	100.6
Soybean	6.65 ± 0.17	66.48 ± 0.8	114.46 ± 0.66	97.0
Citrus leaves	5.10 ± 0.12	51.02 ± 0.4	102.33 ± 0.75	102.6
Corn Stalks	7.79 ± 0.14	78.01 ± 0.4	126.44 ± 0.05	98.0
NIST-SRM 1515 ^a	5.69 ± 0.11	56.96 ± 0.4	106.78 ± 0.56	99.7
NIES-CRM-03 ^b	3.47 ± 0.12	34.74 ± 1.6	85.28 ± 0.30	101.6

Results are expressed as average value ± S.D. (*n* = 3). The samples were spiked with 50 ng ml⁻¹ Cu²⁺. Dilution factor 100 ml g⁻¹.

^a Certified value 5.64 ± 0.23 μg g⁻¹.

^b Certified value 3.5 ± 0.3 μg g⁻¹.

certified materials were employed. In addition, to improve the accuracy of the proposed method with the ordinary samples, the digests of the biological materials were spiked with 50.0 ng ml⁻¹ Cu²⁺, and the recoveries ranged from 97.0 to 101.6%.

Based on the results of Tables 1 and 2 it can be inferred that Cu²⁺ can be successfully determined in natural waters as well as in biological materials, with good accuracy, employing a simple preconcentration system using DAPPS as a sorbent and spectrophotometric detection.

4. Conclusion

A simple flow preconcentration system for the determination of Cu²⁺ using DDTC as chromogenic agent with spectrophotometric detection and DAPPS as a sorbent was successfully employed for the determination of the analyte in biological materials as well as natural water samples. Although the attained preconcentration factor of 36 for Cu²⁺ determination in real samples is not one of the highest values achieved when compared with an off-line column preconcentration, which use 31 of sample solution attaining a preconcentration factor of 300 [17], this value is a little bit better when compared with a flow system with employing reversed-phase C₁₈ as a sorbent, and ethanol as eluent and detection with FAAS, whose preconcentration factor was 19 [31]. In addition, the preconcentration factor attained in this work was also better than that obtained with algae

immobilized in silica gel using ICP-AES as a detector in a flow system, whose value was 13 [25].

The detection limit obtained in this work was 0.84 μg g⁻¹ for biological materials, which is comparable with the detection limit of 0.64 μg g⁻¹ obtained with ETAAS for copper determination in biological materials [41]. It is important to point out that the cost of the implementation of the proposed procedure, employing a homemade flow-injection system was less than US \$4000. It also should be stressed that a graphite furnace spectrometer cost not less than US \$50,000. Therefore, when the flow preconcentration systems coupled to selective sorbents as DAPPS, became widespread, the analysis of low concentration of elements could be attained at any simple analytical laboratory which are numerous in underdeveloped countries.

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