

A BEAD INJECTION SYSTEM FOR COPPER DETERMINATION

Key words: Flow analysis, copper determination, pre-concentration, chelating resin

Claudio C. Oliveira^a, Elias A.G. Zagatto^a, Jaromir Ruzicka^b and Gary D. Christian^{b*}

^a Centro de Energia Nuclear na Agricultura, Universidade de São Paulo
Piracicaba, Brazil

^b University of Washington, Department of Chemistry, Box 351700, Seattle,
Washington 98195-1700, USA.

ABSTRACT

A bead injection system for copper determination is proposed. Chelex-100 resin beads are introduced in the system as a suspension that is trapped in the Jet Ring Cell. The passage of the sample zone by the beads promotes the sorption of Cu(II). When the colorimetric reagent (APDC) perfuses the beads it reacts with copper ions, forming a colored complex that is monitored at 436 nm.

* Corresponding Author: Fax: +1-206-6858665
e-mail: christia@macmail.chem.washington.edu

After the measurement, the spent beads are sent to waste and a new portion of fresh beads is trapped in the system. The bead injection system is versatile and can be used to concentrate analyte in different sample volumes, permitting determinations of a wide range of copper concentrations. The detection limit is $0.5 \mu\text{g l}^{-1}$ with a $500 \mu\text{l}$ sample, and $1.2 \mu\text{g l}^{-1}$ with a $100 \mu\text{l}$ sample.

INTRODUCTION

Flow injection analysis (FIA)¹ permits precise manipulation of solutions for reaction and detection of analytes. Several tedious procedures such as dilutions, development of reactions in beakers and multiple sample manipulations, which may introduce errors in the analysis, can be avoided. It is used to enhance the efficiency of techniques such as atomic absorption² and emission³ spectrometry, and potentiometric and spectrophotometric detection¹. In 1990, a new generation of flow analysis was introduced, sequential injection analysis (SIA)⁴. Here, the confluence points used in FIA were eliminated, as well as the need for different or multiple flow paths for different applications. The system uses a single bi-direction pump to propel solutions connected at a multi-port valve that is used to select the different solutions. The instrument is controlled by means a microcomputer and the injection volumes are based on time and flow rates applied. Sample and reagents are sequentially aspirated into a holding coil, and mixing and reaction occur mainly in a reaction coil on the way to the detector. In the SIA system, different procedures can be carried out in the same flow path, changing only the solutions and volumes with a command from computer.

The sequential injection technique has enabled the bead injection concept⁵. In this approach, a suspension of functionalized beads is introduced in the flow conduit, and trapped in a jet ring cell. Beads act as a solid reagent and sensing medium and can be disposed after each measurement, allowing renewing of the reactive surfaces⁶. This concept has been used mainly in biotechnology

assays such as drug functional discovery⁷ and assays to study bioligand interactions⁸⁻¹⁰ by using spectrophotometric and fluorimetric detection, but has the potential for inorganic ion determinations¹¹.

In the present work, copper is determined, exploiting the bead injection concept. Chelating beads (Chelex - 100) are introduced in the system and the passage of the sample zone by the beads results in Cu(II) retention and accumulation. The subsequent introduction of a colorimetric reagent, ammonium pyrrolidino dithiocarbamate (APDC) promotes the formation of a colored complex that is spectrophotometrically monitored directly on the beads.

EXPERIMENTAL

Apparatus

All fluidic handling was performed using the FIALab-3500 (Alitea USA, Medina, WA) operating in a sequential injection mode with a UV-vis fiber optic CCD spectrophotometer (Ocean Optics, Inc. Dunedin, FL) coupled to the Jet Ring Cell by a fiber optic cable as described elsewhere⁵ (Fig. 1).

Solutions and reagents

The beads used in this work were Chelex-100 resin (200-400 mesh) from Bio-Rad Laboratories, in the sodium form. The bead suspension was prepared by adding 10 ml of water to 0.250 g of the purchased material.

The stock standard Cu(II) solution, 100 mg l⁻¹, was prepared by dissolving 0.0393 g of CuSO₄·5H₂O (Matheson Colemam & Bell, USA) in 15 ml of nitric acid, 1.0 mol l⁻¹ (J.T. Baker), and making up the volume to 100 ml with water. Working solutions were prepared daily by appropriate dilutions of the stock solution and adding nitric acid to obtain 0.014 mol l⁻¹ HNO₃ in the final solution.

The 0.1% (w/v) of ammonium pyrrolidino dithiocarbamate - APDC (K&K Laboratories, USA) solution was prepared dissolving 0.10 g of the

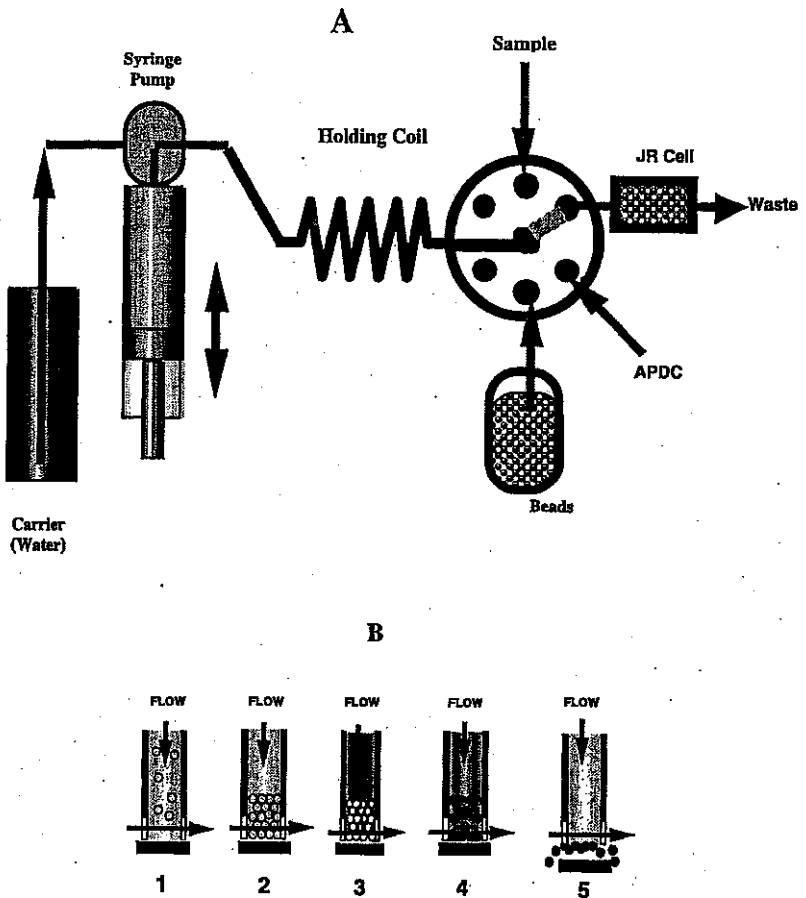


Fig. 1: Sequential injection system. A. Schematic of apparatus.

B. Sequence of flow in Jet ring cell: 1. inject beads; 2. pack beads, measure baseline absorbance; 3. inject sample; 4. inject APDC reagent, measure product absorbance; 5. eject beads to waste.

chemical in 100 ml of water. High purity water (degassed, distilled, de-ionized and treated with Chelex-100 resin) was used as a carrier and to prepare the solutions in all experiments.

Samples

Water samples were preserved in 0.014 mol l⁻¹ of nitric acid and were introduced in the system without any pre-treatment.

Procedure

The sequential injection system as well as the steps for the copper determination are shown in Fig 1A and 1B. Initially, the bead suspension, which is maintained by constant mechanical agitation, is sampled to fill up the Jet Ring cell (Fig. 1B, steps 1 and 2). In the third step, the standard (or sample) is introduced in the system and the beads are perfused to achieve copper retention and concentration. The passage of the colorimetric reagent through the packed beads at 40 $\mu\text{l s}^{-1}$ promotes the reaction between APDC and copper ions, forming the complex that is monitored at 436 nm (step 4). Finally, the spent beads are discarded (step 5) and another cycle is begun.

RESULTS AND DISCUSSION

Sensor response and capacity

It was verified that the Jet Ring Cell could support up to 50 μl of the suspension (1.25 mg of beads). Considering the bead capacity and density (0.4 meq ml⁻¹ and 0.65 g ml⁻¹, respectively), the calculated capacity of the system is 24 $\mu\text{g Cu(II)}$ or 24 ml of a 1 mg l⁻¹ Cu(II) solution. Preliminary experiments demonstrated that when 100 μl of a 200 $\mu\text{g l}^{-1}$ Cu(II) solution flowed by the beads at 10 $\mu\text{l s}^{-1}$ followed by 200 μl of APDC at 40 $\mu\text{l s}^{-1}$, the sensor response was 0.22 A (Fig.2). This corresponds to a concentrated mass of 0.020 μg of Cu(II), and a response of 0.1 A for each 0.0090 μg of copper, and the bead capacity exceeds that needed for sensitive application.

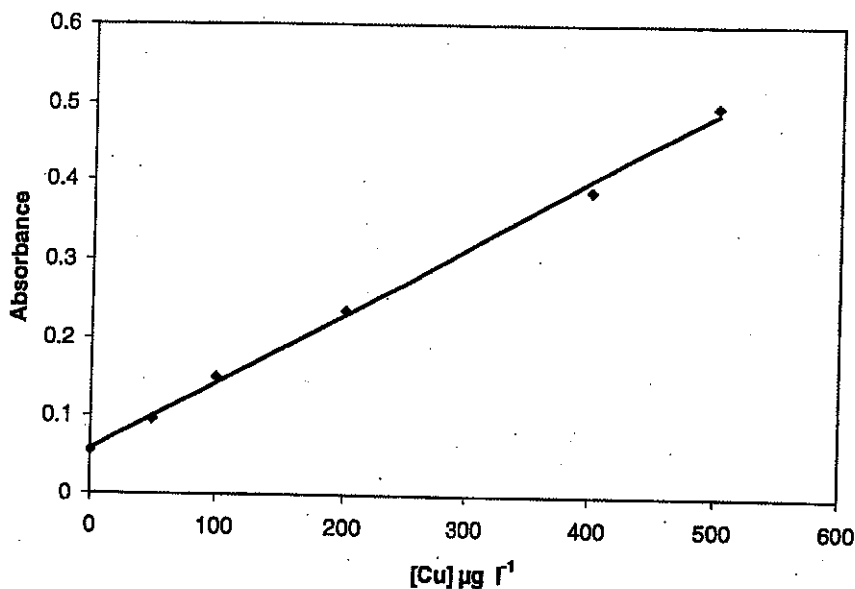


Fig. 2: Analytical curve of copper. Injected volume 100 μl (details in text).

Concentration conditions

The flow rate for the preconcentration step was a very important parameter, since it determines the contact time between copper ions and the beads. In this work to achieve a wide analytical range, two calibration curves were prepared, the first injecting a volume of 500 μl for 0 to 50 $\mu\text{g l}^{-1}$ (Fig.3), and the second injecting a 100 μl volume for 0 to 500 $\mu\text{g l}^{-1}$ (Fig.2). This allowed investigation of the flow rate for the concentration step under both conditions.

When the copper was accumulated on the chelating resin in the absence of buffer solution (in 0.014 mol l^{-1} nitric acid medium), it was observed that sensitivity decreased with increase of the flow rate (Fig.4). The best sensitivity was observed when the flow rate was fixed at 1 or 2 $\mu\text{l s}^{-1}$ which give essentially the same analytical signal; but with this condition the analytical frequency is

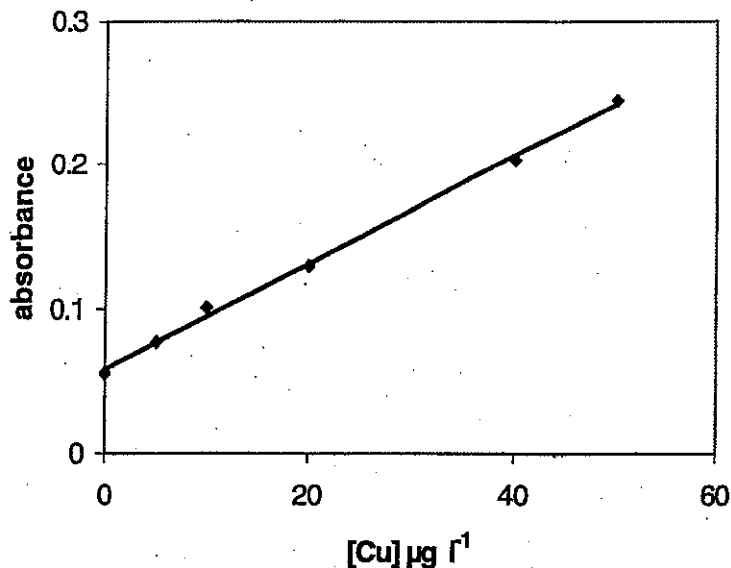


Fig. 3: Analytical curve of copper. Injected volume 500 μl (details in text).

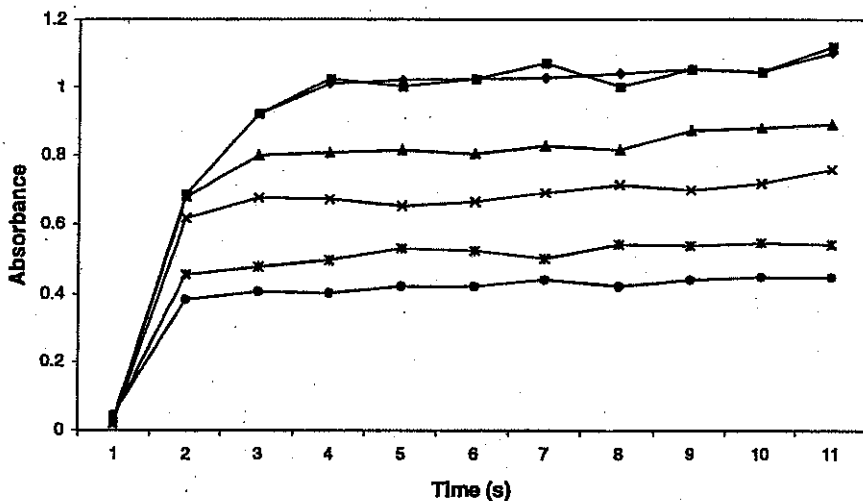


Fig. 4: Effect of flow rate in acid medium. Injected volume of 100 μl of a 500 $\mu\text{g l}^{-1}$ Cu(II) solution, ♦ 1 $\mu\text{l s}^{-1}$, ■ 2 $\mu\text{l s}^{-1}$, ▲ 5 $\mu\text{l s}^{-1}$, x 10 $\mu\text{l s}^{-1}$, * 20 $\mu\text{l s}^{-1}$, • 40 $\mu\text{l s}^{-1}$.

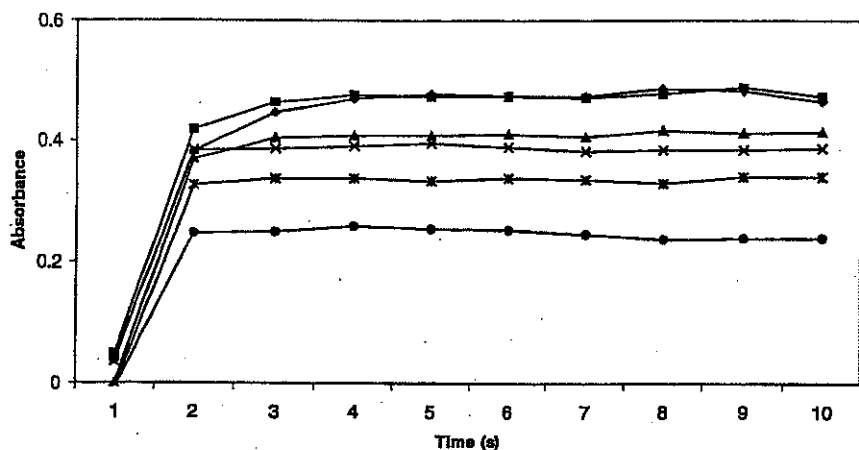


Fig. 5: Effect of flow rate in acetate buffer medium. Injected volume of $100 \mu\text{l}$ of a $500 \mu\text{g l}^{-1}$ Cu(II) solution, ♦ $1 \mu\text{l s}^{-1}$, ■ $2 \mu\text{l s}^{-1}$, ▲ $5 \mu\text{l s}^{-1}$, x $10 \mu\text{l s}^{-1}$, * $20 \mu\text{l s}^{-1}$, • $40 \mu\text{l s}^{-1}$.

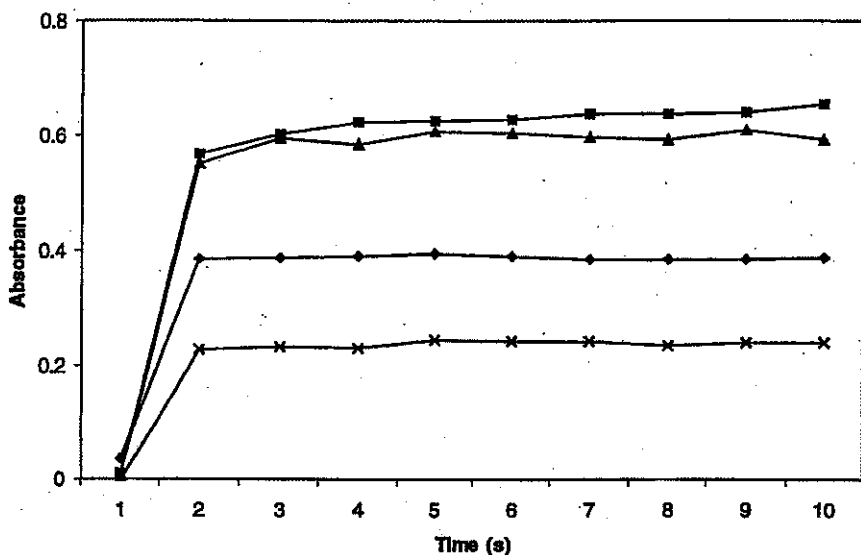


Fig. 6: Effect of ionic strength. Injected volume $100 \mu\text{l}$ of $500 \mu\text{g l}^{-1}$ Cu(II) solution, ■ 0.014 mol l^{-1} in HNO_3 , ▲ 0.014 mol l^{-1} in HNO_3 plus 0.1 mol l^{-1} of sodium chloride, ♦ 0.01 mol l^{-1} of acetate buffer pH 4.5, x 0.01 mol l^{-1} of acetate buffer pH 4.5 plus 0.1 mol l^{-1} of sodium chloride.

very poor, particularly when 500 μl of solution is injected. Hence, 10 $\mu\text{l s}^{-1}$ was chosen as a compromise between analytical frequency and sensitivity. The same experiment was carried out in acetate medium (pH 4.5, 0.1 mol l^{-1} buffer solution) and a loss in sensitivity was found when compared with nitric acid medium (Fig. 4 and 5). This probably occurs because acetate ions compete with the beads for copper ions rather than due to ionic strength increase since, when the ionic strength was increased in acid medium by using an inert salt (NaCl 0.1 mol l^{-1}), the sensitivity was essentially the same as that observed in the acid medium (Fig. 6). Yet, the association of inert salt with acetate buffer caused more reduction in the analytical signal (Fig. 6). For this reason the use of buffer solution is discouraged, particularly with samples that have a high concentration of salts. Samples were preconcentrated directly in acid medium to obtain sensitivity since this does not promote any interference in the reaction between APDC and copper ions and because this reaction occurs in acid medium¹². In these experiments, the beads, following preconcentration, were washed with water prior to the colorimetric reaction.

Another studied parameter was the amount of beads in the system. The analytical signal was reduced with decreased numbers of beads (Fig. 7). While the optical path (length of the column) is decreased, the absorbance would be expected to be the same if the same quantity of copper were adsorbed. The decreased absorbance indicates a decreased amount of copper absorbed, probably in part as the result of decreased interaction between copper ions and the reduced number of beads. This fact confirms the necessity of using a system that can promote very good reproducibility in the selected and delivered bead suspension volume.

Selectivity

It is known that the stability of metal complexes decreases in the order: $\text{Hg(II)} > \text{Cu(II)} > \text{Pb(II)} > \text{Ni(II)} > \text{Cd(II)} > \text{Zn(II)} > \text{Co(II)}$ ¹³. In this work the sorption step was conducted in acid medium, and in this condition the beads

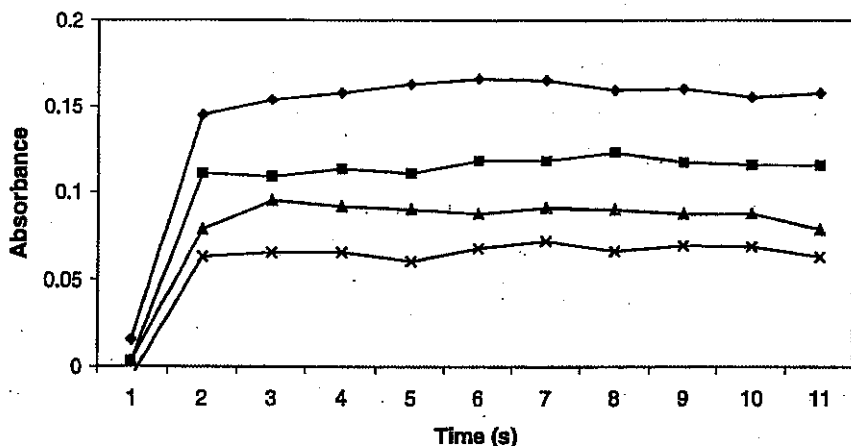


Fig. 7: Effect of the quantity of beads. Injected volume of 500 μl of 40 $\mu\text{g l}^{-1}$ Cu(II) solution, ◆ 1.25 mg, ■ 0.75 mg, ▲ 0.5 mg and x 0.25 mg.

retain only mercury, copper and partially lead ions. In the free form, both metal ions react with APDC¹², but when sorbed on the Chelex-100 beads only Cu(II) reacts. The possible explanation to this fact is that Hg(II) is strongly retained by the beads and can not react with APDC in a short time. The same was observed with Pb(II). In the case of Cu(II), the formation constant of the Cu-APDC complex is sufficiently greater than the Cu-Chelex constant that the reaction occurs almost instantaneously and with high selectivity. Furthermore, Hg(II) and Pb(II) form colorless complexes with APDC, a fact that increases the selectivity of the proposed method.

System characteristics

The following operation conditions were selected as optimum: work with the maximum amount of beads in the Jet Ring Cell (1.25 mg); perform the accumulation step directly in acid medium (0.014 mol l^{-1} of HNO_3) at a flow rate of 10 $\mu\text{l s}^{-1}$; and use 200 μl of APDC flowing at 40 $\mu\text{l s}^{-1}$. With these conditions

TABLE 1

Tap water matrix effects. Recovery of copper ions from tap water samples.

Concentrations expressed in $\mu\text{g l}^{-1}$, with limit of confidence of 95 %.

Sample	[Cu] determined	[Cu] addition	[Cu] found	% Recovery
1	28.2 ± 0.6	20	49.2 ± 1.0	105
2	10.0 ± 0.5	20	9.0 ± 0.7	95
3	31.0 ± 0.7	20	54.0 ± 1.1	115
4	5.4 ± 0.6	20	27.4 ± 0.8	110
5	36.5 ± 0.9	20	55.5 ± 1.2	95
6	20.0 ± 0.8	20	39.4 ± 0.9	97

the analytical curve is linear (Fig. 3), 0 - 50 $\mu\text{g l}^{-1}$, with slope, intercept and correlation coefficients of 0.0037 ($\text{A } \mu\text{g}^{-1} \text{L}$), 0.0586 (A), and 0.997 (r^2), respectively. With a sample volume of 500 μl , the analytical frequency was 45 samples h^{-1} , with a limit of detection of 0.5 $\mu\text{g l}^{-1}$. When the system was operated with a sample volume of 100 μl , the analytical curve characteristics were (0 - 500 $\mu\text{g l}^{-1}$) 0.0008 (A), 0.059 ($\text{A } \mu\text{g}^{-1} \text{L}$), and 0.996 (r^2) as slope, intercept and correlation coefficients (Fig.2), respectively, and the analytical frequency was 70 samples h^{-1} with a detection limit of 1.25 $\mu\text{g l}^{-1}$. Both conditions consume 1.25 mg of beads and 0.20 mg of APDC per determination. When tap water samples are processed in the system, results are not influenced by the matrix and good recovery is obtained (Tab.1). The detection limit can be easily improved by increasing the sample volume, but in this case a loss in analytical frequency occurs. The selected volume will depend on the characteristics of the samples that will be processed and the limit of detection required.

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