Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Talanta 64 (2004) 562–565
Talanta
www.elsevier.com/locate/talanta

Short communication

# A very sensitive flow system for the direct determination of copper in natural waters based on spectrophotometric detection

Juan J. Pinto, Carlos Moreno*, Manuel García-Vargas

*Department of Analytical Chemistry, Faculty of Marine and Environmental Sciences, University of Cádiz, 11510 Puerto Real, Spain*

Received 3 November 2003; received in revised form 29 January 2004; accepted 3 March 2004

---

**Abstract**

A very sensitive flow injection method with spectrophotometric detection has been developed for the on-line determination of copper in natural waters. The method exhibits a limit of detection three times lower than the most sensitive direct spectrophotometric method previously described and then allows the direct and simple in situ determination of copper in most natural waters.

The method was based on the measurement of the absorbance of the coloured complex formed by copper with the chromogenic reagent di-2-pyridyl ketone benzoylhydrazone (dPKBH) in an alkaline medium. This complex presents stoichiometry 1:2 (Cu:dPKBH), and exhibits maximum absorbance at 370 nm. The manifold used was very simple, and consisted of two channels. The first one contained the sample while the second one contained the colorimetric reagent ($3.3 \times 10^{-4}$ M dPKBH in 10% ethanol), in a $1.6 \times 10^{-2}$ M phosphate buffer solution at pH 8. The performance of the system was optimised by using both univariate and modified simplex methodologies. When modified simplex was used, the best signal was obtained for a sample injection volume of 529 μl, a reaction coil length of 1.29 m, and a reagent flow rate of 4.8 ml min$^{-1}$. Under optimum conditions, the response was linear up to 3 mg l$^{-1}$ copper, the equation of the straight line being $y = 0.314x + 5.2 \times 10^{-4}$ ($r^2 = 0.998$). The method allowed a sampling frequency of 40 samples per hour and exhibited a precision of 2.11% (as R.S.D., $n = 11$). The limit of detection was 4.6 μg l$^{-1}$ (calculated as $3s_b/m$, where $s_b$ is the standard deviation of the $y$-intercept and $m$ represents the slope of the straight line), and was therefore more sensitive than all the direct continuous methods reported previously.

The method was successfully applied to the analysis of real water samples, with an average relative error of 5.32%.

*Keywords:* Copper determination; Flow injection; Spectrophotometry; Waters; Trace metals; dPKBH

---

## 1. Introduction

Copper is a heavy metal extensively examined in environmental studies, industrial, biological applications, etc. Many analytical techniques are actually available to analyse copper concentration in samples with different matrices, such as flame atomic absorption spectroscopy [1], electrothermal atomic absorption spectroscopy [2], inductively coupled plasma–atomic emission spectroscopy [3], inductively coupled plasma–mass spectrometry [4] or differential pulse anodic stripping voltammetry [5]. Besides the well-known advantages of these instrumental techniques (precision, accuracy, sensitivity, selectivity, etc.), all of them present a series of disadvantages, such as high investment cost, complexity and difficulty in in situ application. This is especially important in environmental analysis, where very often, results must be obtained in real time, as for example, in on-board studies, where compact systems must be used.

From the practical point of view, to be applied to the in situ analysis of natural waters, a method must be rapid, simple and without preconcentration step. In the case of copper, many times we find concentrations below 5–10 μg l$^{-1}$ and then, limits of detection of about 1–5 μg l$^{-1}$ are required. This sensitivity may be reached only with a preconcentration step, mostly performed by incorporating a preconcentration column to the manifold [6–8]. In most cases, the preconcentration step is not easily applicable to in situ analysis and then, the methods are not useful for practical environmentalists. Actually, there is no direct method with the required sensitivity, and though the flow injection spectrophotometric determination of copper has received much attention, the detection limits were normally higher than 100 μg l$^{-1}$ [9–11]. Recently, limits of detection of 23 μg l$^{-1}$

---

* Corresponding author. Tel.: +34-956-016433; fax: +34-956-016040.
*E-mail address:* carlos.moreno@uca.es (C. Moreno).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.03.009

[12] and 13 μg $l^{-1}$ [13] have been reported, but they are still higher than the values required for direct analysis of copper in some natural waters.

Recently, we described the sensitive spectrophotometric determination of copper using di-2-pyridyl ketone benzoylhydrazone (dPKBH) [14]. Further work has demonstrated the applicability of the reagent for use in flow injection systems focused on the direct determination of copper in water at environmental levels.

## 2. Experimental

### 2.1. Reagents and solutions

Commercial standard solutions of 1000 mg $l^{-1}$ Cu(II) were purchased from Merck (Darmstadt, Germany). All the chemicals used were of analytical reagent grade and purchased from Merck. All the solutions were prepared with MilliQ water (Millipore, USA). The spectrophotometric reagent dPKBH was synthesised, according to the procedure outlined by García-Vargas et al. [15]. Thus, equimolar amounts of di-2-pyridyl ketone and benzoylhydrazide were mixed in ethanol, and a few drops of concentrated hydrochloric acid were added. The mixture was refluxed for 1 h, and after cooling, MilliQ water was added to a 1:3 volume ratio ($V_{\mathrm{org}}$:$V_{\mathrm{aq}}$). Several drops of a sodium hydroxide solution were added to obtain a slightly acid solution. The solid obtained was re-crystallized twice, dissolved in ethanol, and reprecipitated with water. Stock solutions of dPKBH (0.4% w/v) were prepared by dissolving the synthesised reagent in ethanol. The FIA reagent was $3.25 \times 10^{-4}$ mol $l^{-1}$ dPKBH in ethanol (final concentration 10%) at pH 8, kept by a $1.65 \times 10^{-2}$ mol $l^{-1}$ phosphate buffer solution.

### 2.2. Instruments

The flow injection manifold consisted on a Perimax 12 four-path peristaltic pump (Spetec, Germany) equipped with Tygon tubing, which was used to manipulate the flows of two channels (reagent and sample solutions), a Model 1106 injection valve (Omnifit, UK), and a PU 8750 UV-Vis spectrophotometer (Philips, The Netherlands) equipped with a quartz flow cell with a 10 mm pathlength (Hellma, Germany). Transport lines and reaction coils were made using 0.8 mm i.d. PTFE tubing (Omnifit, UK). Connections were made of polypropylene (Omnifit, UK).

Acidity was measured with a Model 2001 pH-meter provided with a combined pH electrode (Crison, Spain).

### 2.3. Procedure

The chemical conditions used were those reported above and were selected from the results obtained in our previous study [14]. To ensure the highest sensitivity of the determination method, the optimisation of the FI manifold was performed in two steps: first, a univariate pre-optimisation procedure was done by varying the sample injection volume (66–956 μl), reaction coil length (1–4 m), and reagent flow rate (1.3–6.5 ml $\min^{-1}$). Then, a second optimisation process was carried out to find optimum conditions. This process was performed by applying a modified simplex methodology, by using the software MultiSimplex 98 (MultiSimplex AB, Sweden) [16].

The optimisation studies were carried out with synthetic sample solutions containing 0.1 mg $l^{-1}$ Cu(II), and by measuring the absorbance of the Cu-dPKBH complex formed, at 370 nm. For each experimental point, the increment of absorbance (sample minus blank) was calculated as an average of, at least, three replicates. Once optimised, the proposed method was applied to the determination of copper in real natural waters samples.

## 3. Results and discussion

### 3.1. Optimisation of FI system

#### 3.1.1. Univariate method

Table 1 shows the range over which the variables were studied and the values selected as optimum. These optimum values were chosen mainly by the highest signal and in terms of reproducibility, time and reagent consumption.

The spectrophotometric signal increased with injection volume up to 750–800 μl, then the absorbance was almost constant. When reaction coil length and reagent flow rate were varied, very similar curves were obtained, with an initial increase of the signal and, after reaching a maximum zone, the signal decreased. This behaviour may be explained in terms of the opposite effects caused by mixing and dispersion of the sample into the reagent stream.

#### 3.1.2. Modified simplex method

In the second procedure, based on simplex optimisation, the three variables were varied simultaneously. The conditions of the initial simplex (shown in Table 2) were selected by using the knowledge obtained from the univariate pre-optimisation. A total of 38 different experiments were required to decide the optimum conditions. The criterion chosen for stopping the search was based on the measurement of response variation due to the simplex. This variation

Table 1
Study ranges and optimum conditions selected from univariate optimisation of FIA manifold

| Variable | Range | Optimum value |
|---|---|---|
| Sample injection volume (μl) | 66–956 | 837 |
| Reaction coil length (m) | 1–4 | 1.73 |
| Reagent flow rate (ml $\min^{-1}$) | 1.3–6.5 | 5.3 |

Table 2
Initial conditions (vertexes 1–4), optimum conditions (vertex 32), and last experiment (vertex 38) in modified simplex optimisation of FIA manifold

| Vertex | I.V. (μl) | R.C. (m) | F.R. (ml $min^{-1}$) | *A* |
|---|---|---|---|---|
| 1 | 416 | 2.23 | 2.0 | 0.046 |
| 2 | 416 | 1.73 | 3.4 | 0.132 |
| 3 | 516 | 1.73 | 2.0 | 0.062 |
| 4 | 516 | 2.23 | 3.4 | 0.119 |
| ... | ... | ... | ... | ... |
| 32 | 529 | 1.29 | 4.8 | 0.255 |
| ... | ... | ... | ... | ... |
| 38 | 457 | 0.41 | 18.3 | 0.241 |

I.V.: sample injection volume; R.C.: reaction coil length; F.R.: reagent flow rate; and A: absorbance.

was estimated by means of sample variance of all the responses of each simplex. A small response variation means that differences between response values at the vertexes are due only to random error. This estimation was carried out by calculating the *F*-values for the ratio of simplex variance and a variance of the method, which was experimentally calculated [17]. As shown in Table 2, trial 32 gave the highest response value, the optimum conditions being: length of reaction coil: 1.29 m, sample injection volume 529 μl, and reagent flow rate 4.8 ml $min^{-1}$.

As expected, by using simplex optimisation a better selection of experimental conditions could be done and then, an improvement in the spectrophotometric signals was obtained.

Under optimum conditions, a sampling rate of 40 samples per hour was obtained, and Beer's law was obeyed up to 3 mg $l^{-1}$. The equation of the straight line was $y = 0.314(\pm 0.028)x + 5.2(\pm 0.5) \times 10^{-4}$ ($r^2 = 0.998$). The confidence intervals for slope and *y*-intercept were estimated as *ts*, where *t* is the Student-*t* ($P = 0.05$, $n - 2$) and *s* is the corresponding standard deviation. The precision of the method was established from the measurement of 11 samples containing 0.1 mg $l^{-1}$ Cu(II), giving a relative standard deviation of 2.11%.

The limit of detection, calculated as $3s_b/m$ (where $s_b$ is the standard deviation of the *y*-intercept and *m* represent the slope of the straight line), was 4.6 μg $l^{-1}$. It must be noted that this method provides the highest sensitivity among the continuous methods based on direct determinations, and then allows the analysis of copper in most natural waters avoiding a preconcentration step. This fact implies a significant advance for the in situ measurement of copper in natural water. In this sense, Table 3 shows a comparison of our method with some of the similar methods previously reported for the direct determination of copper by flow injection at the ppb-level, including some fluorometric methods. As can be observed in Table 3, the new method presents a limit of detection more than twice lower than the most sensitive one among the previous methods, and as a consequence is the only one allowing quantification of copper concentrations as low as 10–20 μg $l^{-1}$.

### 3.2. *Study of interferences*

Potential interferences caused by several cations in the form of chlorides, nitrates or sulphates and anions as potassium, sodium or ammonium salts were studied. Several cations interfered (variation of signal higher than 5%) at higher ($Mg^{2+}$, $Al^{3+}$, $Pb^{2+}$, $Ag^{+}$), similar ($Mn^{2+}$) or even lower ($Zn^{2+}$, $Ni^{2+}$, $Co^{2+}$, $Cd^{2+}$, $Hg^{2+}$) molar concentration than copper concentration. Although in many real samples most of the interfering species appear at lower concentration than copper, we studied the use of masking agents to avoid the potential interferences. Best results were obtained with the addition of ammonium fluoride ($3.2 \times 10^{-3}$ M), which decreased or even removed the interferences of $Mg^{2+}$, $Al^{3+}$ and $Mn^{2+}$ and sodium citrate ($2.0 \times 10^{-3}$ M) which was an appropriate masking agent for $Zn^{2+}$, $Cd^{2+}$ and $Ni^{2+}$.

### 3.3. *Application*

The new method was applied to the on-line determination of copper in two real water samples. The first one consisted on a tap water sample containing 13.21 μg $l^{-1}$ Cu, 30.90 μg $l^{-1}$ Zn, 41.07 μg $l^{-1}$ Fe, 146.66 μg $l^{-1}$ Al and 2.56 μg $l^{-1}$ Mn (at-line measured by ET-AAS). This sample was directly analysed, by triplicate, with the proposed method. The second sample was river water containing 542 μg $l^{-1}$ Cu, 590 μg $l^{-1}$ Zn, 604 μg $l^{-1}$ Fe and 63.8 μg $l^{-1}$ Pb (off-line measured by ICP-AES). This sam-

Table 3
Comparison of the analytical methods allowing direct determination of copper by flow injection at the ppb-levels

| Sensitivity (μg $l^{-1}$) | Technique | Reagent | Reference |
|---|---|---|---|
| 680[a] | Spectrophotometry | Nitroso-R salt | Pucharat et al. [9] |
| 500[b] | Spectrophotometry | Cuprizone | Liu et al. [10] |
| 500[b] | Fluorometry | Thiamine | Pérez Ruiz et al. [18] |
| 130[a] | Spectrophotometry | Cuprizone | Chimpalee et al. [11] |
| 23[a] | Spectrophotometry | Diethyldithiocarbamate | Cassella [12] |
| 13[a] | Spectrophotometry | Cuprizone | Rumori and Cerdà [13] |
| 10[a] | Fluorometry | 5-(4-Chlorophenylazo)-8-aminoquinoline | Cao et al. [19] |
| 4.6[a] | Spectrophotometry | Di-2-pyridyl ketone benzoylhydrazone | This work |

[a] Expressed as limit of detection.

[b] Expressed as lower value of linear range.

Table 4
Analysis of copper in different real samples

| Sample | Reference method ($\mu g\, l^{-1}$) | This method[a] ($\mu g\, l^{-1}$) | $\varepsilon_r$ (%) |
|---|---|---|---|
| Tap water | 13.21 (±0.44) | 12.22 (±0.20) | −7.49 |
| River water | 542 (±42) | 525 (±72) | −3.14 |

Results expressed as concentration ± standard deviation. $\varepsilon_r$: relative error.
[a] Triplicate analysis.

ple was previously 10 times diluted and analysed with the proposed method by triplicate. To avoid the potential interferences caused by other cations, sodium citrate ($2.0 \times 10^{-3}$ M) and ammonium fluoride ($3.2 \times 10^{-3}$ M) were added to the samples.

Table 4 shows the average concentration measured for each sample and their corresponding standard deviations. As can be observed, the results obtained are in good agreement with those values used as reference. The accuracy of the results was tested by applying the paired *t*-test, which confirmed the absence of systematic errors at the 0.05 level.

## References

[1] D.T.E. Hunt, A.L. Wilson, The Chemical Analysis of Water. The Royal Society of Chemistry, Oxford, 1986, p. 398.
[2] A.E. Watson, Nucl. Active 30 (1984) 31.
[3] T.M. Dymott, in: G. Subramaniam (Ed.), Quality Assurance in Environmental Monitoring, VCH, Weinheim, 1995, p. 114.
[4] J.W. McLaren, in: K.E. Jarnis, A.L. Gray, R.S. Houk (Eds.), Handbook of Inductively Coupled Plasma Mass Spectrometry, Blackie & Son Ltd., Glasgow, 1992, p. 266.
[5] G. Gillain, G. Duyckaerts, A. Disteche, Anal. Chim. Acta 106 (1979) 23.
[6] F.C. Camargo, E.A.G. Zagatto, C.C. Oliveira, Anal. Sci. 14 (1999) 565.
[7] A. Asan, I. Isildak, M. Andac, F. Yilmaz, Talanta 60 (2003) 861.
[8] A. Ali, H. Shen, X. Gu, G.M. Xu, Y.X. Ye, X.F. Yin, Lab. Robotics Automat. 12 (2000) 97.
[9] B. Pucharat, S. Liawruangrath, P. Sooksamiti, S. Rattanaphani, D. Buddhasukh, Anal. Sci. 17 (2002) 443.
[10] J.F. Liu, Y.D. Feng, G.B. Jiang, J. AOC Int. 84 (2001) 1179.
[11] N. Chimpalee, D. Chimpalee, S. Srithawepoon, T. Patjarut, D.T. Burns, Anal. Chim. Acta 304 (1995) 97.
[12] R.J. Cassella, Microchem. J 72 (2002) 17.
[13] P. Rumori, V. Cerdà, Anal. Chim. Acta 486 (2003) 227.
[14] J.J. Pinto, C. Moreno, M. García-Vargas, Anal. Bioanal. Chem. 373 (2002) 844.
[15] M. García-Vargas, M. Belizón, M.P. Hernández-Artiga, C. Martínez, J.A. Pérez-Bustamante, Appl. Spectrosc. 40 (1986) 1058.
[16] Multisimplex 98, User's Guide and Reference, Multisimplex AB, Karlskrona, Sweden, 1998.
[17] C.K. Bayne, I.B. Rubin, Practical Experimental Designs and Optimization Methods for Chemists, VCH Publishers Inc., FL, 1986, p. 128.
[18] T. Pérez Ruiz, C. Martínez Lozano, V. Tomás, I. Ibarra, Talanta 39 (1992) 907.
[19] Q.E. Cao, K.T. Wang, Z.D. Hu, Q.H. Xu, Food Chem. 65 (1999) 405.