

## Determination of Trace Amounts of Copper using a Flow-Through Spectrophotometric Sensor

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### Abstract

A flow-through spectrophotometric sensor for the determination of copper based on the retention of Cu(I)-1,2-cyclohexanedione thiosemicarbazone complex on C<sub>18</sub>-bonded beads packed in a flow-cell was developed. The method is highly selective for copper; it features the detection limit of 50 ng ml<sup>-1</sup>, and a linear range from at least 0.1 to 10 µg ml<sup>-1</sup>. The method is subject to very few interferences and is more sensitive than the batch procedure. The proposed method was applied to the determination of copper in catalysts and alloys.

**Keywords:** Copper, flow-through sensor, spectrophotometry

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### 1. Introduction

Copper is widely distributed in nature. The analysis of copper-containing materials has a vast literature. This is due to widespread interest in copper contents in several branches of science (e.g. medicine, biology, industrial chemistry and geology).

Flow injection methods offer great advantages such as flexibility, speed, high sampling rate and wide applicability in many fields. Based on flow-injection principles, and with spectroscopic detection, one of the current trends is the immobilization of the components of a chemical reaction in a flow cell placed in the light path of a molecular spectroscopic detector. Modified flow-cells located in conventional or unconventional detector have been used to integrate reaction (retention) and detection of analytes. Any of the components of an analytical reaction (analytes, reagents catalysts and products) can be permanently or temporarily retained on a support packed in the flow-cell [1-3].

Yoshimura [4] reported the first system based on the measurement of the absorbance of copper ion by using a cation exchange resin located in the flow cell as support.

Analytical derivatization reactions can be implemented in integrated sorption-detection system by using reagent immobilized (bonded or adsorbed) on support that is subsequently placed in the flow cell.

This approach has been applied to the spectrophotometric and spectrofluorimetric determination of metal ions. Cu(II) was determined at the ng ml<sup>-1</sup> level by immobilizing 4-(2-pyridylazo)resorcinol on a cation-exchange resin packed in a spectrophotometric flow cell [5]. Be(II), Ca(II), Al(III) and Zn(II) were determined by means of a spectrophotometric or spectrofluorimetric flow-through sensor containing reagents immobilized on different resins [6-10].

Flow-through sensors based on the temporary immobilization of a solid support of reaction products formed previously in the continuous manifold offer a wide range of applications. Ion-exchanger phase absorptiometry was developed for trace amounts of copper in water with 4,7-diphenyl-2,9-dimethyl-1,10-phenanthroline disulphonate and applied to investigate storm runoff of karst groundwater[11]. Bi(III) was determined by forming iodide complexes and retaining them in a flow-through cell containing Sephadex anion exchanger as support[12]. The temporary retention of the product formed between Cr(VI) and diphenylcarbazide on a cation-exchange resin was exploited for the determination of this ion[13]. Fe(III)

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can be determined in water and wine by forming Fe(III)-thiocyanate complex and retention on Dowex 1-X2-200 anion exchanger located in the flow cell[9]. Also, Co(II) was determined in pharmaceutical preparation by using retention of Co(II)-pyridoxal 4-phenyl 3-thiosemicarbazone complex on C<sub>18</sub> as sorbent[14].

Thiosemicarbazones generally react as chelating ligands for transition metal ions by bonding through the sulfur and hydrazine nitrogen atoms and their analytical uses have been exhaustively reviewed in the last few years[15-17].

The aim of this work lies in the recently developed flow-through sensor technology by which retention and detection are integrated in a FI system. In this case, successive passage of the complex formed between Cu(II) and 1,2-cyclohexanedione dithiosemicarbazone(CHDT)[18](previously formed in the flowing stream) and elution through the flow cell packed with C<sub>18</sub> and continuous photometric monitoring of the process provide the analytical information needed to determine copper. The method is more selective and sensitive than that previously reported, with a detection limit of 0.05 µg ml<sup>-1</sup>.

## 2. Experimental

### 2.1. Reagents

All chemicals used were of analytical-reagent grade and solutions were prepared using high-purity water from a Millipore Milli-Q water purification system.

Stock standard solution (1.000 g l<sup>-1</sup>) of copper(II) was prepared from the sulfate, and standardized complexometrically. Working standards solutions were prepared by appropriate dilution daily as required.

A 0.1 % (m/v) solution of 1,2 cyclohexanedione dithiosemicarbazone (CHDT) was prepared in N,N-dimethylformamide (DMF). The reagent was synthesized following a procedure described elsewhere[18].

A pH 4.8 buffer was prepared by mixing 30 ml of 0.2 M sodium acetate and 20 ml of 0.2 M acetic acid and diluting to 100 ml with deionized water. DMF was used as eluent. C<sub>18</sub> material from a Sep-Pak C<sub>18</sub> cartridge (Millipore Waters, Milford, MA, USA) was used for the retention and detection of copper complex in the flow cell.

### 2.2. Instrumentation and procedure

A Hewlett-Packard 8452A diode-array detector interfaced to HP Vectra QS/ 165 personal computer which delivered results through an HP LaserJet printer was used. The flow manifold consisted of a Gilson Minipuls-3 peristaltic pump, a Rheodyne Type 50 six-port rotary valve was used as injection valve or selection valve, and a Hellma (Jamaica, NY, USA) OS 0.200 flow cell with a 2 mm optical path length.

A schematic diagram of the FI system is shown in Fig.1. It operated as follows: Two different configurations were tested. In the first configuration, the sample was merged with a buffer stream at a flow rate of 0.5 ml min<sup>-1</sup>. The stream was merged at point M with the reagent solution (0.1 % CHDT in DMF) at a flow rate of 0.6 ml min<sup>-1</sup>. The reaction product formed (Cu-CHDT complex) in mixing coil R (250 cm x 0.5 mm i.d.) filled the loop of the injection valve L. Meanwhile, DMF was passed through of the flow cell, packed with C<sub>18</sub>. When the injection valve is changed to inject position, the complex was retained on C<sub>18</sub> packed in the flow cell (preconcentration step). As the plug tail reached the detector (160 s after injection), the concentrated retained reaction product was readily eluted with DMF, thereby restoring the baseline.

A switching valve can be used instead of an injection valve, in order to ensure a higher reproducibility and sampling rate and lower sample consumption. In this case, when SV is changed to position 2, the absorbance signal increased to a constant value, and when the SV was switched to position 1, DMF was passed through flow cell, so the signal was rapidly returned to its baseline.

### 2.3. Sample preparation

The certified materials (CRMs) analysed to determine the accuracy of the proposed procedure were: Bureau of Analysed Samples LTD 20b Aluminium Alloy, catalyst that was supplied by the Spanish oil product manufacturer CEPSA and steels that were supplied by CENIM. These samples were prepared according to the following procedures.

*Al Alloy:* Dissolve a known amount of accurately weighed sample (0.1 g) in about 30 ml of aqua regia; evaporate the resulting solution to dryness and add 1 ml of hydrochloric acid (1 + 1); then dilute with distilled water to 50 ml.

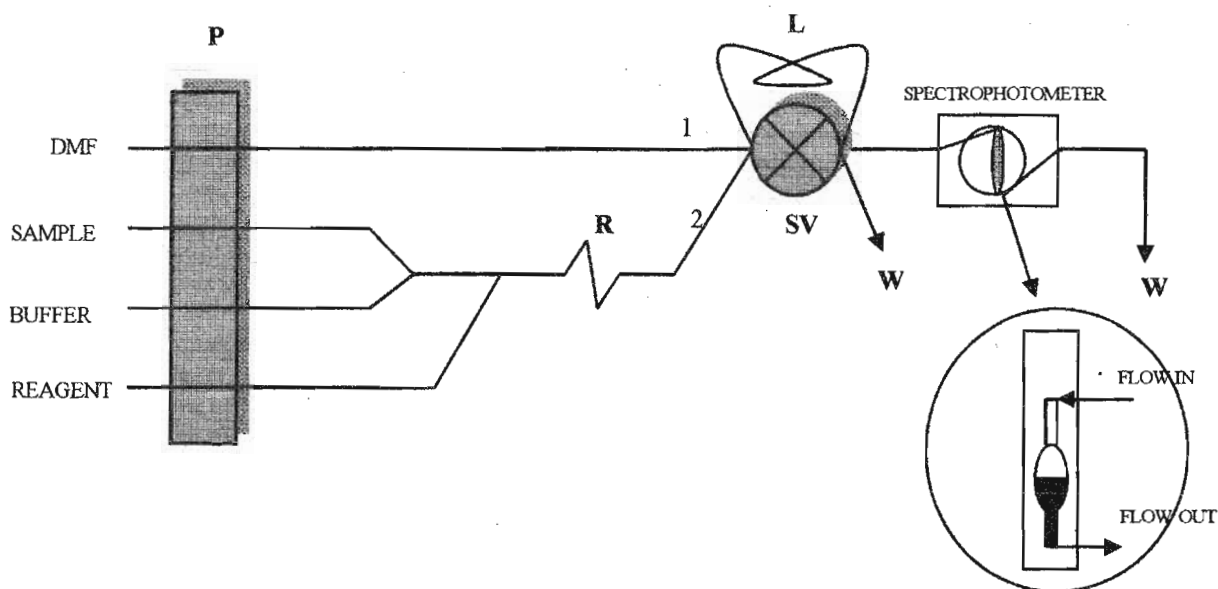


Fig. 1 Schematic diagram for the determination of copper. P, peristaltic pump; R, mixing coil (length 250 cm, i.d. 0.5 mm); L, injection loop; SV, selection valve; W, waste

**Catalyst :** Dissolve 0.1 g of sample in 4 ml of aqua regia which was added a known amount of standard solution of copper; evaporate the resulting solution to dryness and to dilute to 50 ml.

**Steels:** Dissolve 0.5 g of each sample in 40 ml of aqua regia. The silica residue is filtered and excess acid is removed by heating and then to dilute to 50 ml

Analysis of these samples were made adding 10 ml of EDTA 0.1 M to each one in order to eliminate interferences of other ions.

### 3. Results and discussion

Copper forms an orange 1:1 complex with CHDT ( $\lambda = 462 \text{ nm}$ ,  $\epsilon = 5.7 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ ). The complex is stable over the pH range 3.8 to 10. From experimental evidence it was concluded that the reagent forms the orange complex with copper(I). For this purpose, ascorbic acid was also used as a reducing agent and the Cu-CHDT complex showed the same spectrum with and without reductor agent. This reaction can be carried out with the reagent or complex retained on a sorbent material. Thus in preliminary assays several sorbent materials were tested in order to find the most appropriate for this purpose. So cationic exchange resins (Dowex, Amberlite, SP-Sephadex) were assayed; however, neither the reagent nor the complex was retained. Other sorbent materials, such as

aluminium oxide, silicagel and  $C_{18}$  were tested, but only the latter effectively retained both the reagent and the complex.

Several eluents were tested ( $\text{HNO}_3$ ,  $\text{HCl}$ ,  $\text{HNO}_3 + \text{HCl}$ , DMF) from these, DMF proved to be the most efficient for removing the complex from the  $C_{18}$  without damaging it.

The next task was to select the most advantageous experimental procedure. If the reagent was retained on  $C_{18}$  first and then metal ion (in acetic/acetate medium) was passed through it, the manifold provides a poor response because the  $C_{18}$  was saturated with the reagent, so the subsequent passage of the sample through the sensor gave an inadequately sensitive signal. The complex was thus formed in the flow and then retained on  $C_{18}$ . The reactant addition sequence providing the highest sensitivity was sample, buffer and CHDT.

#### 3.1. Selection of the FI manifold

We first optimized the normal FI method. If the sample is injected into a channel with buffer or reagent, near-zero absorbance was obtained when  $1 \mu\text{g ml}^{-1}$  of copper was used. So, we assay an other FI manifold where sample was merged with a buffer stream and then merged with the reagent stream, and the injection valve

was used to introduce the reaction product formed in the reactor to the detector. Also a selection valve can be used in order to ensure a higher sampling rate and lower sample and reagent consumption. The manifold selected is schematically depicted in Figure 1. The FI system was assembled from 0.5 mm i.d. tubing.

### 3.2. Optimization of variables

The variables affecting the performance of the proposed method were optimized by the univariate method. By plotting the analytical signal (maximum of the FIA recording) against the value of the variable concerned we obtained maxima at the values listed as optimal in Table 1 for the reactor length and flow rate. The distance between M and N must be as shorter as possible in order to avoid the dilution of the sample in the buffer. R was varied from 50 to 300 cm. A 250 cm reactor length was selected as optimum to ensure reaction development.

Table 1 Optimum values of flow injection variables

Sample flow rate	1.3 ml min <sup>-1</sup>
Reagent flow rate	0.6 ml min <sup>-1</sup>
Buffer flow rate	0.5 ml min <sup>-1</sup>
Eluent flow rate	0.5 ml min <sup>-1</sup>
R dimensions	250 cm x 0.5 mm i.d.

Chemical variables studied included sample pH and reagent concentration. Buffer acetic acid/ sodium acetate of different pH was passed through the second channel. There is no effect between pH 4 - 5. A 4.8 pH buffer was used in subsequent experiments. On the other hand, different CHDT concentrations were tested between 0.05 - 0.2 % (m/v) dissolved in DMF. From these, maximum signal was obtained using 0.1 % of CHDT.

All above described was optimized using selection valve. If injection valve was used, the analytical signal also increased on increasing the injected volume. A 2 ml injection loop volume was used in order to ensure approximately 60 s of constant absorbance before elution.

Scans were performed in the kinetic mode at 462 nm for 300 s, in cycles of 1 s with an integrating time of 0.5 s. Fig. 2 shows typical recording obtained and the point for analytical measurements (marked with arrowheads).

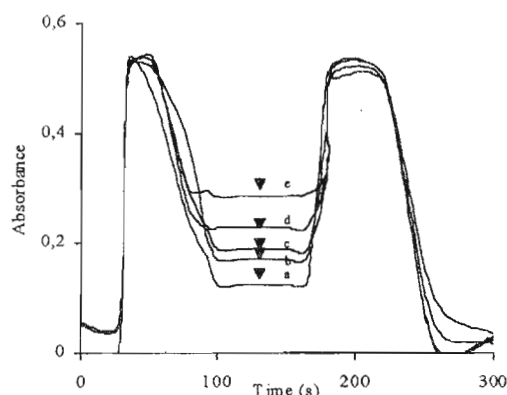


Fig. 2. FI peaks yielded by (a) blank, (b) 0.1, (c) 1.0 (d) 5.0 and (e) 10.0  $\mu\text{g ml}^{-1}$  of copper

### 3.3. Calibration curve, detection limit and repeatability

In order to establish the optimal range for the determination of copper by the proposed method, several standard solutions of copper(II) were passed by the channel of sample into the flow system under the optimum experimental conditions. From the data obtained, a calibration curve could be expressed by a linear regression equation:

$$A = 1.15 \times 10^{-2} C (\mu\text{g ml}^{-1}) + 0.17 \quad (n=5, r=0.998)$$

where  $A$  is the measured signal in absorbance;  $C$  the concentration of the analyte;  $n$  the number of standard samples tested;  $r$  the regression coefficient. The absorbance obtained was proportional to copper concentration from 0.1 to 10  $\mu\text{g ml}^{-1}$ ; the precision or repeatability, expressed as relative standard deviation (r.s.d.) was 2.04 % ( $n=10$ ) for 0.2  $\mu\text{g ml}^{-1}$  and 1.04 % ( $n=10$ ) for 1  $\mu\text{g ml}^{-1}$  of copper, respectively. The detection limit defined as the concentration of analyte giving signal equivalent to three times the standard deviation of the blank plus the net blank intensity was calculated to be 50 ng ml<sup>-1</sup>.

### 3.4. Interferences

Some major inorganic species usually present in the real samples were studied as potential interferents with the copper determination in order to testify the interference eliminating capability by the proposed method. For this purpose, variable amounts of the ionic species tested were added to 3  $\mu\text{g ml}^{-1}$  solution of Cu(II) up to a maximum interferent copper ratio of 200 : 1 (M/M); if any compound was found to interfere, the

ratio was gradually lowered until the interference disappeared. The tolerated limits are given in Table 2. Ions such as Co(II), Fe(II), Zn(II), Cd(II), Ni(II) that were tolerated to less concentrations, can be increased their concentrations with aids of masking agent. So, 10 ml of EDTA 0.1 M was used as masking agent. As can be seen, the proposed method is very selective.

Table 2 Tolerance to foreign ions in the determination of Cu

Tolerance ratio (M/M) <sup>a</sup>	Ion or species
> 200	Pb(II), As(III), Al(III), Zr(IV), Mn(II), Ca(II), Sr(II), Ba(II), Mg(II), Li(I), CN <sup>-</sup> , Mo(VI), SCN <sup>-</sup> , F <sup>-</sup> , Cl <sup>-</sup> , Br <sup>-</sup> , EDTA, Co(II)(EDTA as masking agent), Fe(II)(EDTA as masking agent), Zn(II)(EDTA as masking agent), Cd(II)(EDTA as masking agent), Ni(II)(EDTA as masking agent)

<sup>a</sup> Maximum concentration tolerated for the interferent with respect to the analyte ( $\mu\text{g ml}^{-1}$  in each case)

### 3.5. Applications

In order to test the applicability and matrix interferences of the proposed method in the analysis of real samples, the method was applied to the determination of copper in alloys, catalyst and steels. These samples were prepared and analysed as described under *Experimental*. The results obtained using a calibration graph are shown in Table 3. These results indicate that the method would be effective for the analysis of samples of similar complexity.

Table 3 Determination of copper in real samples

Sample	Certified %	Added %	Found*
Al alloy	4.10		4.05 ± 0.01
Catalyst		2.15	2.25 ± 0.01
Steel 1		0.287	0.286 ± 0.003
Steel 2		0.493	0.479 ± 0.016

\* Mean ± Standard deviation, n= 3

## 4. Conclusions

Copper can be determined at the nanogram per milliliter level by using an integrated continuous retention-detection flow approach. A comparison of the method proposed here with its conventional counterpart [18] shows that the present method is faster and simpler, as reflected in the fact that all the steps involved are performed in the flow system, requires far less sample and reagent, and has limit of determination 20 times lower.

The most salient advantages of the proposed method are as follows: reduced human participation (automation capability); higher throughput than the batch procedure; high selectivity; reusability of the sensor so it can be regenerated by elution of the retained reaction product.

*The authors thank the Dirección General de Investigación Científica y Técnica (DGICYT) for supporting this study (Project PB96-0702) and also the Junta de Andalucía.*

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(Received January 12, 2000)

(Accepted April 17, 2000)