

Short communication

# On-line preconcentration/determination of copper in parenteral solutions using activated carbon by inductively coupled plasma optical emission spectrometry

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## Abstract

A method for the on-line preconcentration of copper using a minicolumn packed with activated carbon and its subsequent determination by inductively coupled plasma optical emission spectrometry (ICP-OES) coupled with flow injection (FI) was studied. In order to determine the copper concentration present in parenteral solutions; it was retained on activated carbon (AC) at pH 9.5. A sensitivity enrichment factor of 30-fold was obtained with respect to the copper determination by ICP-OES without preconcentration. The detection limit for the preconcentration of 25 ml of sample was  $0.1 \mu\text{g l}^{-1}$ . The precision for the ten replicate determinations at the  $2.5 \mu\text{g l}^{-1}$  Cu level was 3.0% relative standard deviation (R.S.D.), calculated with the peak heights. The calibration graph using the preconcentration method for copper species was linear with a correlation coefficient of 0.9996 at levels near the detection limits up to at least  $200 \mu\text{g l}^{-1}$ . The method was successfully applied to the determination of copper in parenteral solutions.

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## 1. Introduction

Total parenteral nutrition (TPN) can be defined as the procedure by which all required nutrients are administered intravenously. In those individuals who are unable to intake food by an oral or enteral route, parenteral nutrition is required to sustain life. In addition to providing a positive caloric balance with optimized infusion of protein, carbohydrate, lipids, electrolytes, and vitamins, appropriate supplementation of essential trace elements (cobalt, chromium, copper, iodine, iron, manganese, molybdenum, selenium, and zinc) must also be included and customized to each patient's clinical situation [1].

The presence of trace metals in parenteral nutrition assumes a special relevance because some of them are potentially dangerous or even very toxic. Therefore, the control of the finished product regarding metal content is advisable [2–4].

Copper is an important component of several oxidative enzymes in human metabolism. Its serum and urine concentrations in various conditions, such as Wilson's disease (hepatolenticular degeneration) may be useful as indicators of Cu utilization [5].

Manifestations of copper deficiency in patients on unsupplemented hyperalimentation are well documented [6–9]. Initial clinical signs are neutropenia and hypochromic microcytic anemia that and unresponsive to iron supplementation. In the infant maintained on TPN, copper deficiency also resulted in the skeletal abnormalities of osteoporosis and retarded bone growth [10].

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There are several techniques which have been used for determination of copper at low concentrations, such as inductively coupled plasma optical emission spectrometry (ICP-OES) [11], electrothermal atomic absorption spectrometry (ETAAS) [12,13], X-ray fluorescence (XRF) [14], flame atomic absorption spectrometry (FAAS) [15,16], inductively coupled plasma-mass spectrometry (ICP-MS) [17,18], capillary electrophoresis [19], among others.

Although the ICP-OES or AAS are the most used techniques in the determination of traces of copper, the low level of copper concentration in parenteral solutions is not compatible with the detection limits of these techniques. In order to achieve accurate, reliable and sensitive results, preconcentration is needed when the concentration of the elements in the sample are too low to be determined directly by the analytical technique.

Various preconcentration techniques for the determination of copper have been proposed, including chelation, sorption on any sorbent and cloud point extraction [11–13,15,16,18,19]. Since its introduction in analytical chemistry, activated carbon (AC) has been used as a collector, permitting the preconcentration and separation of low levels of analytes in several matrices [20–22]. The mechanism of sorption on AC is still under investigation. The most common models used for heavy metals adsorption are the classic empirical adsorption (e.g. Langmuir and Freundlich equations). The major advantage of these models is their simplicity; however, the models fail to describe accurately the sorption equilibrium under varying conditions, such as pH and ionic strength. The adsorption equilibrium studies have revealed that pH is the dominant parameter controlling the adsorption [23].

In the present work, a method for on-line preconcentration of copper coupled to ICP-OES determination is proposed. Copper was retained by sorption on the minicolumn packed with activated carbon in absence of complexing reagent, under the form of Cu(II), the pH values adjustment of the solution suffices to retain copper(II) ion. The determination was performed by ICP-OES associated with a FI methodology.

## 2. Experimental

### 2.1. Reagents

The activated carbon (Merck, Darmstadt, Germany, 50–70 mesh) was used after pretreatment with acid (activated carbon was heated with 10% (v/v) hydrochloric acid for 30 min and then with 10% (v/v) nitric acid for 20 min and finally washed with deionized water at neutral pH was reached).

A stock standard solution of 1000 mg l<sup>-1</sup> Cu(II) was prepared from copper oxide (Fluka) and diluted to a final volume of 1000 ml.

Table 1  
ICP-OES instrumental parameters employed for copper determination

Forward power (kW)	1.0
RF generator (MHz)	40.68
Nebulizer	Glass, meinhard
Coolant gas flow rate (l min <sup>-1</sup> )	8.5
Auxiliary gas flow rate (l min <sup>-1</sup> )	1.0
Carrier gas flow rate (l min <sup>-1</sup> )	0.5
Solution uptake rate (ml min <sup>-1</sup> )	1.5
Observation height (above load coil) (mm)	15

A buffer solution was prepared by diluting a 3.0 mol l<sup>-1</sup> ammonium hydroxide solution adjusted to pH 9.5 with a hydrochloric acid solution.

Ultrapure water (18 MΩ cm) was obtained from EASY pure RF (Barnsted, IA, USA). All the reagents were of analytical-reagent grade and the presence of copper was not detected within the working range.

### 2.2. Instrumental

The measurements were performed with an ICP-OES [BAIRD (Bedford, MA, USA) ICP 2070]. The 1m-Czerny Turner monochromator had an holographic grating with 1800 grooves mm<sup>-1</sup>. The ICP operating conditions are listed in Table 1. A Minipulse peristaltic pump [Gilson (Villiers, Le-Bell, France)] was used. Sample injection was achieved using a Rheodine model 50 4-way (Cotati, CA, USA). A conical minicolumn (40 mm length, 4.5 mm internal upper-diameter and 1.5 mm internal lower-diameter) was used as the AC holder. Pump tubes-Tygon type (Ismatec, Cole-Parmer Instrument Company, Niles, IL, USA) were employed to propel the sample and eluent. The 327.396 nm emission line was used and measurements of the FI system were expressed as peak-height, which was corrected against the reagents blank.

### 2.3. Column preparation

The conical minicolumn was prepared following the procedure reported in previous papers [20–22]. The average lifetime of column in more than 300 cycles. All columns prepared by this way shown good reproducibility. It was proved by precision of the signals obtained for different columns.

### 2.4. Preconcentration procedure and determination

Before loading, the column was conditioned for preconcentration at the correct pH with the buffer diluted (1:10) solution, valve V<sub>1</sub> in position B (Fig. 1). The sample (25 ml) buffered to pH 9.5 was then loaded on the AC at flow rate of 20.0 ml min<sup>-1</sup> with valve V<sub>1</sub> in position S and valve V<sub>2</sub> in load position (a). Finally, valve V<sub>2</sub> was switched to the injection position (b) and the retained metal was eluted with 20% (v/v) nitric acid directly in pneumatic nebulizer and subsequently to the ICP-OES. The operating conditions were established and the determination was carried out.

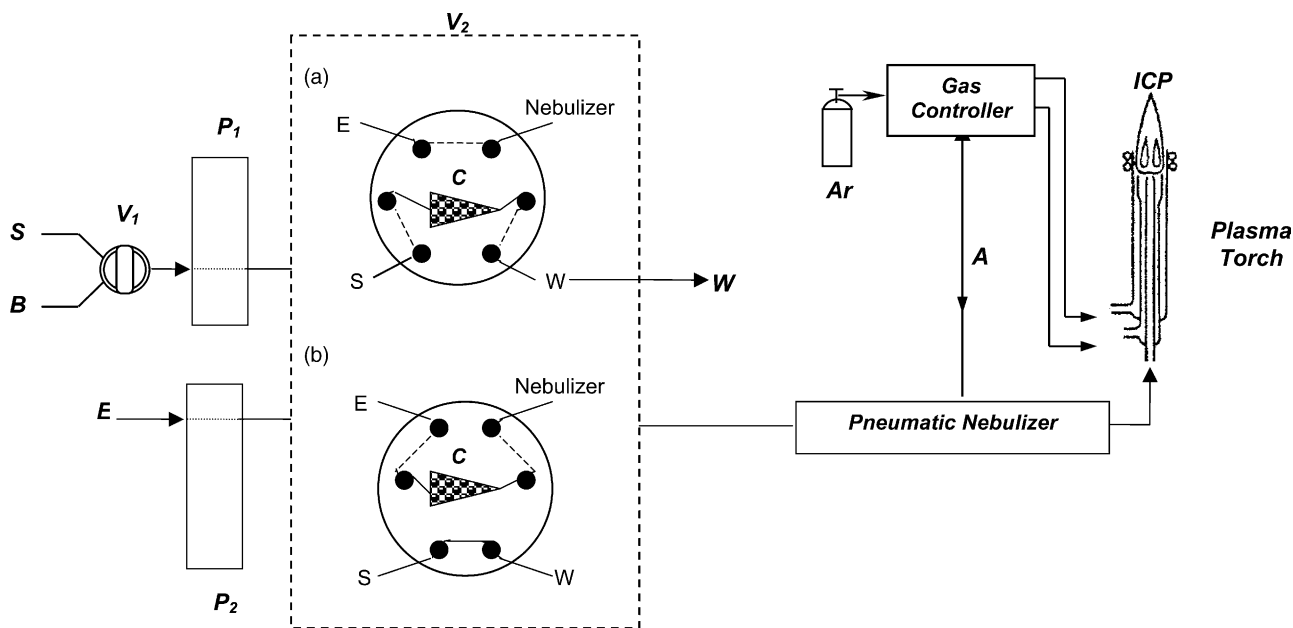


Fig. 1. Schematic diagram of the instrumental setup. S, sample; B, buffer diluted; E, eluent; W, waste; P<sub>1</sub> and P<sub>2</sub>, peristaltic pumps; M, minicolumn; V<sub>1</sub>, two-way valve, V<sub>2</sub>, load injection valve ((a) load position; (b) injection position).

### 3. Results and discussion

#### 3.1. Effect of parameters controlling the preconcentration procedure

In order to optimize the sorption conditions for the retention of the copper on AC, the copper signal was monitored by measuring it with ICP-OES while changing the pH of the solution that passes through the conical minicolumn packed with AC (with small additions of NaOH or HCl, respectively). The optimal pH values were in the range of 8.5–11.0. Accordingly, the selected pH was 9.5.

The sample flow rate through the column packed with AC is a very important parameter, since this is one of steps that controls the time of analysis. An important advantage of our system is the high sample flow rate reached. We could verify that with flow rates up to 20 ml min<sup>-1</sup> there are no effect on the analyte recovery, at higher flow rates the recovery decreases.

On the other hand, in order to elute the analyte sorbed on AC, nitric acid was chosen as eluent. The copper signal was monitored by measuring it ICP-OES while changing the nitric acid concentration. We could verify that with nitric acid concentrations between 20 and 50% (v/v) there is no effect on the analyte recovery. A 20% (v/v) nitric acid concentration was used in subsequent studies.

It is well known that the column design strongly influences the performance of preconcentration system [24]. For this reason, the proposed method was applied to a conical minicolumn —4.5 mm internal upper-diameter and 1.5 mm internal lower-diameter; packed with activated carbon. From our studies, we could verify that the conical column perfor-

mance was much better than that the classical column due to the conical design produces a diminishing of the dispersion; the improvement was 80%.

#### 3.2. Interferences

The effects of representative potential interference species (at the concentration levels at which they may occur in the studied sample) were tested. Co<sup>2+</sup>, Cr<sup>3+</sup>, Zn<sup>2+</sup>, Pb<sup>2+</sup>, Cd<sup>2+</sup>, Ni<sup>2+</sup>, Mn<sup>2+</sup>, and Fe<sup>3+</sup> could be tolerated up to at least 500 µg l<sup>-1</sup>. Commonly encountered such alkali and alkaline elements are not retained on the AC under working conditions. On the other hand, anions such as CO<sub>3</sub><sup>2-</sup>, F<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, Cl<sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, malonate and ascorbate could be tolerated up to at least 1000 µg l<sup>-1</sup>.

#### 3.3. Recovery study

In order to demonstrate the validity of this method, 250 ml of parenteral solution were collected and divided into twenty portions of 25 ml each. The proposed method was applied to ten portions and the average quantity of Cu was taken as a base value. Then increasing quantities of Cu were added to the other aliquots of sample and the analyte was determined by the same method. As shown in Table 2, the recovery values are between 96.7 and 102.0%. The results were compared with the *t*-test and no significant differences were observed at 95% confidence level [25].

#### 3.4. Analytical performance

The overall time required for preconcentration of 25 ml of sample (1.25 min, at flow rate of 20 ml min<sup>-1</sup>), elution

Table 2

Recovery study

Aliquots	Base value ( $\mu\text{g l}^{-1}$ )	Quantity of Cu added ( $\mu\text{g l}^{-1}$ )	Quantity of Cu found ( $\mu\text{g l}^{-1}$ )	Recovery (%) <sup>a</sup>
1–6	–	0.00	$5.6 \pm 0.3$	–
7	5.6	1.0	6.6	100.0
8	5.6	3.0	8.5	96.7
9	5.6	5.0	10.7	102.0
10	5.6	8.0	13.5	98.8

<sup>a</sup>  $100 \times [(\text{found} - \text{base})/\text{added}]$ .

Table 3

Concentration of copper in parenteral solutions samples (95% confidence interval;  $n = 6$ )

Sample	Cu concentration ( $\mu\text{g l}^{-1}$ )
1 <sup>a</sup>	$11.7 \pm 0.4$
2 <sup>b</sup>	$5.5 \pm 0.3$
3 <sup>c</sup>	$5.6 \pm 0.3$
4 <sup>d</sup>	$6.0 \pm 0.4$

<sup>a</sup> Ringer physiological solution.<sup>b</sup> KCl parenteral solution.<sup>c</sup> NaCl parenteral solution.<sup>d</sup> Isotonic dextrose 5% physiological solution.

(0.4 min, at flow rate of  $2 \text{ ml min}^{-1}$ ), and washing and conditioning (0.4 min) was about 2.05 min; hence, throughput was approximately 29 samples  $\text{h}^{-1}$ . An enrichment factor of 30-fold was obtained with respect to the copper determination in parenteral solutions by ICP-OES without preconcentration.

The reproducibility of the preconcentration method was evaluated by passing 25 ml of standard solution at  $2.5 \mu\text{g l}^{-1}$  copper level through the minicolumn and repeating this procedure 10 times. The relative standard deviation (R.S.D.) was 3.0%, calculated from the peaks heights obtained. The calibration graph using the preconcentration system (the standard solutions were preconcentrated on the activated carbon) for copper was linear with a correlation coefficient of 0.9996 at levels close to the detection limit up to at least  $200 \mu\text{g l}^{-1}$ . The detection limit (DL) was calculated as the amount of copper required to yield a net peak that was equal to three times the standard deviation of the background signal ( $3\sigma$ ). The value of DL obtained for the preconcentration of 25 ml of aqueous solutions of Cu was  $0.1 \mu\text{g l}^{-1}$ . Finally, the results of the method applied to the copper determination in parenteral solutions samples are shown in Table 3. The concentrations were in the range  $5.5\text{--}11.7 \mu\text{g l}^{-1}$  of copper. The results obtained are in good agreement with those of Almeida et al. [26].

#### 4. Conclusions

The work described in this paper has shown that adequate sensitivity and accuracy can be attained using an on-line pre-

concentration system with a FI-ICP-OES method. The coupling of an on-line system with FI-ICP-OES increases the speed of the preconcentration and analysis process, and reduces sample consumption and contamination risks. Besides, the manifold studied permitted to reach sample flow rates as higher as  $20 \text{ ml min}^{-1}$ . The proposed system of preconcentration associated with ICP-OES allowed the Cu determination in parenteral solutions at concentrations as low as  $\mu\text{g l}^{-1}$ .

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